	L #	Hits	Search Text	DBs	Time Stamp
1	L1	472	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2003/02/10 08:32
2	L2	4827	trehalose	USPAT; US-PGPUB	2003/02/10 08:33
3	L3	49	1 and 2	USPAT; US-PGPUB	2003/02/10 08:33
4	L4	128	non adj reducing adj saccharide$1	USPAT; US-PGPUB	2003/02/10 08:39
5	L5	21	1 and 4	USPAT; US-PGPUB	2003/02/10 08:40
6	L6	4851	$trehalose	USPAT; US-PGPUB	2003/02/10 08:41
7	L7	49	1 and 6	USPAT; US-PGPUB	2003/02/10 08:41

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	472	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2003/02/10 08:32
2	L2	4827	trehalose	USPAT; US-PGPUB	2003/02/10 08:33
3	L3	49	1 and 2	USPAT; US-PGPUB	2003/02/10 08:33

PGPUB-DOCUMENT-NUMBER: 20030017573

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030017573 A1

TITLE: Polymerase kappa compositions and methods thereof

PUBLICATION-DATE: January 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Friedberg, Errol C.	Dallas	TX	US	
Gerlach, Valerie	Branford	CT	US	
Feaver, William J.	Branford	CT	US	

APPL-NO: 09/ 971101

DATE FILED: October 4, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60238289 20001004 US

US-CL-CURRENT: 435/226,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

The present invention concerns compositions and methods involving mammalian polymerase kappa, an enzyme with limited fidelity and moderate processivity. Methods of modulating polymerase kappa activity, such as inhibiting or reducing its activity, as a means of effecting a cancer treatment or preventative agent are provided, both by itself and in combination with other anti-cancer therapies. Also described are methods of screening involving assaying for polymerase kappa activity or expression, in addition to methods of screening for modulators of polymerase kappa to identify anti-cancer compounds.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/238,289, filed Oct. 4, 2000, the entire disclosure of which is specifically incorporated herein by reference. The government may own rights in the present invention pursuant to grant numbers CA 75733 and CA69029 from the National Cancer Institute.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0054] The early stages of the evolutionary history of the UmuC/DinB

superfamily of TLS-associated polymerases are uncertain because of horizontal gene transfer and lineage-specific gene loss. The importance of these modes of evolution is supported both by the patchy distribution of these polymerases in bacteria and archaea (with only one archaeal member identified so far in the crenarchaeon **Sulfolobus** solfataricus (Kuleava et al., 1996)), and by the location of the umuC genes on plasmids and the uvrX gene in a bacteriophage. It appears that at least one gene coding for this type of polymerase was present at the base of the eukaryotic crown group, with an early duplication resulting in the emergence of the Rev1 and Rad30 (Pol.eta./.iota.) families. A subsequent duplication, probably at an early stage of metazoan evolution, resulted in the divergence of pol .eta. and pol .iota..

Detail Description Paragraph - DETX:

[0109] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion also is contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20030003452

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030003452 A1

TITLE: High fidelity reverse transcriptases and uses thereof

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Potter, Robert Jason	Frederick	MD	US	
Rosenthal, Kim	Laytonsville	MD	US	

APPL-NO: 09/ 808124

DATE FILED: March 15, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60189454 20000315 US

US-CL-CURRENT: 435/6,435/199 ,435/320.1 ,435/325 ,435/69.1 ,435/91.2 ,536/23.2

ABSTRACT:

The invention relates to reverse transcriptases which have increased fidelity (or reduced misincorporation rate) and/or terminal deoxynucleotidyl transferase activity. In particular, the invention relates to a method of making such reverse transcriptases by modifying or mutating specified positions in the reverse transcriptases. The invention also relates to nucleic acid molecules containing the genes encoding the reverse trancriptases of the invention, to host cells containing such nucleic acid molecules and to methods to make the reverse trancriptases using the host cells. The reverse transcriptases of the invention are particularly suited for nucleic acid synthesis, sequencing, amplification and cDNA synthesis.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 60/189,454, filed Mar. 15, 2000, the contents of which are incorporated by reference herein in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0111] A variety of DNA polymerases are useful in accordance with the present invention. Such polymerases include, but are not limited to, Thermus thermophilus (Tth) DNA polymerase, Thermus aquaticus (Taq) DNA polymerase, Thermotoga neapolitana (Tne) DNA polymerase, Thermotoga maritima (Tma) DNA polymerase, Thermococcus litoralis (Tli or VENT.TM.) DNA polymerase, Pyrococcus furiosis (Pfu) DNA polymerase, DEEPVENT.TM. DNA polymerase, Pyrococcus woosii (Pwo) DNA polymerase, Bacillus sterothermophilus (Bst) DNA polymerase, Bacillus caldophilus (Bca) DNA polymerase, **Sulfolobus acidocaldarius** (Sac) DNA polymerase, Thermoplasma acidophilum (Tac) DNA polymerase, Thermus flavus (Tfl/Tub) DNA polymerase, Thermus ruber (Tru) DNA polymerase, Thermus brockianus (DYNAZYME.TM.) DNA polymerase, Methanobacterium thermoautotrophicum (Mth) DNA polymerase, Mycobacterium spp. DNA polymerase (Mtb, Mlep), and mutants, variants and derivatives thereof.

Detail Description Paragraph - DETX:

[0116] In addition to the enzyme components, the present compositions preferably comprise one or more buffers and cofactors necessary for synthesis of a nucleic acid molecule such as a cDNA molecule. Particularly preferred buffers for use in forming the present compositions are the acetate, sulfate, hydrochloride, phosphate or free acid forms of Tris-(hydroxymethyl)aminomethane (TRIS.RTM.), although alternative buffers of the same approximate ionic strength and pKa as TRIS.RTM. may be used with equivalent results. In addition to the buffer salts, cofactor salts such as those of potassium (preferably potassium chloride or potassium acetate) and magnesium (preferably magnesium chloride or magnesium acetate) are included in the compositions. Addition of one or more carbohydrates and/or sugars to the compositions and/or synthesis reaction mixtures may also be advantageous, to support enhanced stability of the compositions and/or reaction mixtures upon storage. Preferred such carbohydrates or sugars for inclusion in the compositions and/or synthesis reaction mixtures of the invention include, but are not limited to, sucrose, **trehalose,** and the like. Furthermore, such carbohydrates and/or sugars may be added to the storage buffers for the enzymes used in the production of the enzyme compositions and kits of the invention. Such carbohydrates and/or sugars are commercially available from a number of sources, including Sigma (St. Louis, Mo.).

Detail Description Paragraph - DETX:

[0122] In another aspect, the compositions and reverse transcriptases of the invention may be prepared and stored in dry form in the presence of one or more carbohydrates, sugars, or synthetic polymers. Preferred carbohydrates, sugars or polymers for the preparation of dried compositions or reverse transcriptases include, but are not limited to, sucrose, **trehalose,** and polyvinylpyrrolidone (PVP) or combinations thereof. See, e.g., U.S. Pat. Nos. 5,098,893, 4,891,319, and 5,556,771, the disclosures of which are entirely incorporated herein by reference. Such dried compositions and enzymes may be stored at various temperatures for extended times without significant deterioration of enzymes or components of the compositions of the invention. Preferably, the dried reverse transcriptases or compositions are stored at 4.degree. C. or at -20.degree. C.

PGPUB-DOCUMENT-NUMBER: 20020197698

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197698 A1

TITLE: Novel amylases

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hagihara, Hiroshi	Haga-gun		JP	
Kitayama, Kaori	Haga-gun		JP	
Hayashi, Yasuhiro	Haga-gun		JP	
Igarashi, Kazuaki	Haga-gun		JP	
Endo, Keiji	Haga-gun		JP	
Ozaki, Katsuya	Haga-gun		JP	

APPL-NO: 10/ 136272

DATE FILED: May 2, 2002

RELATED-US-APPL-DATA:

child 10136272 A1 20020502 parent division-of 09465519 19991216 US GRANTED parent-patent 6403355 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	10-362487	1998JP-10-362487	December 21, 1998
JP	10-362488	1998JP-10-362488	December 21, 1998

US-CL-CURRENT: 435/201,435/320.1 ,435/325 ,435/69.1 ,536/23.2

ABSTRACT:

Liquefying alkaline amylases, each having residual activity not less than 70% when treated at pH 10 and 45.degree. C. for 30 minutes in the presence of 1 to 100 mM of EDTA or EGTA, are described. Also described are detergents comprising these amylases. In comparison with conventional amylases for detergents, the liquefying alkaline amylases of this invention have a high chelating-agent resisting performance.

--------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0006] Among the liquefying amylases known to date, a liquefying .alpha.-amylase (WO90/11352) derived from the strain belonging to Pyrococcus sp. and an .alpha.-amylase (WO96/02633) which is derived from the strain belonging to **Sulfolobus** sp. and is effective in the liquefying step of a starch are free from the influence from a chelating agent. These enzymes however have the optimum acting pH in a range of 4 to 6 and 2.5 to 4.5, respectively and do not act in the alkaline range so that they are not suited as a component of a detergent.

Detail Description Table CWU - DETL:

1 TABLE 1 Strain KSM-K36 Stain KSM-K38 (a) Results of microscopic The strains K36 and K38 are bacilli having a size of 1.0 to 1.2 .mu.m .times. observation 2.4 to 5.4 .mu.m and 1.0 to 1.2 .mu.m .times. 1.8 to 3.8 .mu.m, respectively. They form an oval endospore (1.0 to 1.2 .mu.m .times. 1.2 to 1.4 micron) at the center or near the end of the cell. Positive in the Gram's stain. Having no acid resistance. (b) Growth in various media Since the present strain is alkaliphilic, 0.5% sodium carbonate is added to the medium employed in the following tests. Nutrient agar plate culture Good growth is observed. The . Good growth is observed. The colony has a circular shape. It colony has a circular shape. It has a flat surface, but a rough has a flat surface and a smooth periphery. The color of the periphery. The color of the colony is pale earthlike color. colony is yellowish brown. Nutrient agar slant culture Growth is observed. Growth is observed. Nutrient broth culture Growth is observed. Growth is observed. Nutrient-gelatin stab culture Good growth is observed. No Good growth is observed. No liquefaction of gelatin is liquefaction of gelatin is observed. observed. Litmus milk No change is observed. No change is observed. (c) Physiological properties Reduction of a nitrate and Reduction of a nitrate is positive. Reduction of a nitrate is positive. denitrification reaction Denitrification reaction is Denitrification reaction is negative, negative. MR test Owing to the alkaline medium, Owing to the alkaline medium, judgment is impossible. judgment is impossible. V-P test Negative. Negative. Formation of indole Negative. Negative. Formation of hydrogen nitride Negative. Negative. Hydrolysis of starch Positive. Positive. Assimilation of citric acid It grows on a Christensen's It grows on a Christensen's medium, but not on a Koser's medium, but not on a Koser's medium and Simmon's medium. medium and Simmon's medium. Assimilation of an inorganic It assimilates a nitrate but not an It assimilates a nitrate but not an nitrogen source ammonium salt. ammonium salt. Formation of a colorant Formation of a pale yellow Negative. colorant on King's B medium. Urease Negative. Negative. Oxidase Negative. Negative. Catalase Positive. Positive. Range for growth Temperature range for growth is Temperature range for growth is 15 to 40.degree. C. The optimum growth 15 to 40.degree. C. The optimum growth temperature ranges from 30 to temperature is 30.degree. C. 37.degree. C. The pH range for growth is 9.0 to The pH range for growth is 8.0 to 11.0. The optimum growth pH is 11.0. The optimum growth pH is similar to the above. pH 10.0 to 11.0. Behavior to oxygen Aerophilic. Aerophilic. O-F test No growth is observed. No growth is observed. Assimilation of saccharides Assimilated are D-galactose, D-xylose, L-arabinose, lactose, glycerin, meribiose, libose, D-glucose, D-mannose, maltose, sucrose, **trehalose,** D-mannitol, starch, raffinose and D-fructose. Growth on a salt-containing Grown at a salt concentration of 12%, but no

growth at a salt medium concentration of 15%.

PGPUB-DOCUMENT-NUMBER: 20020197605

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197605 A1

TITLE: Novel Polynucleotides

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nakagawa, Satoshi	Tokyo		JP	
Mizoguchi, Hiroshi	Tokyo		JP	
Ando, Seiko	Tokyo		JP	
Hayashi, Mikiro	Tokyo		JP	
Ochiai, Keiko	Tokyo		JP	
Yokoi, Haruhiko	Tokyo		JP	
Tateishi, Naoko	Tokyo		JP	
Senoh, Akihiro	Tokyo		JP	
Ikeda, Masato	Tokyo		JP	
Ozaki, Akio	Hofu-shi		JP	

APPL-NO: 09/ 738626

DATE FILED: December 18, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	P. HEI 11-377484	1999JP-P. HEI 11-377484	December 16, 1999
JP	P. 2000-159162	2000JP-P. 2000-159162	April 7, 2000
JP	P. 2000-280988	2000JP-P. 2000-280988	August 3, 2000

US-CL-CURRENT: 435/6,435/287.2 ,435/91.2

ABSTRACT:

Novel polynucleotides derived from microorganisms belonging to coryneform bacteria and fragments thereof, polypeptides encoded by the polynucleotides and fragments thereof, polynucleotide arrays comprising the polynucleotides and fragments thereof, recording media in which the nucleotide sequences of the polynucleotide and fragments thereof have been recorded which are readable in a computer, and use of them.

---------- KWIC ---------

Detail Description Table CWU - DETL:

A3(2) 45.0 56.0 95 putative ATP/GTP-binding protein 1347 4847 1281714 1281262 453 sp:YQJC_BACSU Bacillus subtilis yqjC 35.8 68.7 134 hypothetical protein 1348 4848 1281794 1282105 312 sp:YC20_MYCTU Mycobacterium tuberculosis 54.5 79.2 101 hypothetical protein H37Rv Rv1898 1349 4849 1282194 1283114 921 sp:YD24_MYCTU Mycobacterium tuberculosis 37.9 71.4 301 thioredoxin H37Rv Rv1324 1350 4850 1283324 1284466 1143 gp:ECO237695_3 Escherichia coli K12 ssuD 50.3 74.3 366 FMNH2-dependent aliphatic sulfonate monooxygenase 1351 4851 1284517 1285284 768 sp:SSUC_ECOLI Escherichia coli K12 ssuC 40.8 75.8 240 alphatic sulfonates transport permease protein 1352 4852 1285302 1286030 729 sp:SSUB_ECOLI Escherichia coli K12 ssuB 50.4 72.8 228 alphatic sulfonates transport permease protein 1353 4853 1286043 1286999 957 sp:SSUA_ECOLI Escherichia coli K12 ssuA 35.1 62.1 311 sulfonate binding protein precursor 1354 4854 1289473 1287281 2193 sp:GLGB_ECOLI Mycobacterium tuberculosis 46.1 72.7 710 1,4-alpha-glucan branching enzyme H37Rv Rv1326c glgB (glycogen branching enzyme) 1355 4855 1291007 1289514 1494 sp:AMY3_DICTH Dictyoglomus thermophilum 22.9 50.5 467 alpha-amylase amyC 1356 4856 1291026 1291373 348 1357 4857 1291699 1292577 879 sp:FEPC_ECOLI Escherichia coli K12 fepC 31.8 87.6 211 ferric enterobactin transport ATP- binding protein or ABC transport ATP-binding protein 1358 4858 1293222 1294025 804 pir:C70860 Mycobacterium tuberculosis 39.6 68.5 260 hypothetical protein H37Rv Rv3040c 1359 4859 1294151 1295206 1056 pir:H70859 Mycobacterium tuberculosis 43.1 70.0 367 hypothetical protein H37Rv Rv3037c 1360 4860 1295047 1294436 612 1361 4861 1295435 1296220 786 sp:FIXA_RHIME Rhizobium meliloti fixA 31.2 64.8 244 electron transfer flavoprotein beta- subunit 1362 4862 1296253 1297203 951 sp:FIXB_RHIME Rhizobium meliloti fixB 33.1 61.8 335 electron transfer flavoprotein alpha subunit for various dehydrogenases 1363 4863 1296479 1297093 615 1364 4864 1297212 1298339 1128 sp:NIFS_AZOVI Azotobacter vinelandii nifS 35.2 67.7 375 nitrogenase cofactor sythesis protein 1365 4865 1298653 1298342 312 1366 4866 1300145 1299000 1146 sp:Y4ME_RHISN Rhizobium sp. NGR234 plasmid 29.5 55.7 397 hypothetical protein pNGR234a y4mE 1367 4867 1300369 1300145 225 sp.Y4MF_RHISN Rhizobium sp. NGR234 plasmid 47.5 76.3 59 transcriptional regulator pNGR234a Y4mF 1368 4868 1300552 1301055 504 sp:YHBS_ECOLI Escherichia coli K12 MG1655 34.8 55.3 181 acetyltransferase yhbS 1369 4869 1301929 1300988 942 1370 4870 1303123 1301975 1149 1371 4871 1303299 1303694 396 1372 4872 1303829 1304923 1095 pir:C70858 Mycobacterium tuberculosis 61.8 80.9 361 tRNA (5-methylaminomethyl-2- H37Rv Rv3024c thiouridylate)-methyltransferase 1373 4873 1304536 1303883 654 1374 4874 1304932 1305921 990 pir:B70857 Mycobacterium tuberculosis 33.7 66.0 332 hypothetical protein H37Rv Rv3015c 1375 4875 1307384 1305924 1461 sp:TCMA_STRGA Streptomyces glaucescens tcmA 30.2 65.8 500 tetracenomycin C resistance and export protin 1376 4876 1308196 1307462 735 1377 4877 1308330 1310369 2040 sp:DNLJ_RHOMR Rhodothermus marinus dnlJ 42.8 70.6 677 DNA ligase (polydeoxyribonucleotide synthase [NAD+] 1378 4878 1311097 1310435 663 pir:H70856 Mycobacterium tuberculosis 40.0 70.9 220 hypothetical protein H37Rv Rv3013 1379 4879 1311320 1311616 297 sp:GATC_STRCO Streptomyces coelicolor A3(2) 53.0 64.0 97 glutamyl-tRNA(Gln) gatC amidotransferase subunit C 1380 4880 1311625 1313115 1491 sp:GATA_MYCTU Mycobacterium tuberculosis 74.0 83.0 484 glutamyl-tRNA(Gln) H37Rv gatA amidotransferase subunit A 1381 4881 1313270 1314118 849 sp:VIUB_VIBVU Vibrio vulnificus viuB 28.1 54.0 263 vibriobactin utilization protein / iron- chelator utilization protein 1382 4882 1314775 1314470 306 gp:SCE6_24 Streptomyces coelicolor A3(2) 46.9 79.2 96 hypothetical membrane protein SCE6.24 1383 4883 1315013 1316083 1071 sp:PFP_AMYME Amycolatopsis methanolica pfp 54.8 77.9 358

pyrophosphate-fructose 6- phosphate 1-phosphotransrefase 1384 4884 1315954 1315325 630 1385 4885 1316338 1317444 1107 sp:CCPA_BACME Bacillus megaterium ccpA 31.4 31.4 328 glucose-resistance amylase regulator (catabolite control protein) 1386 4886 1317434 1319005 1572 sp:RBSA_ECOLI Escherichia coli K12 rbsA 44.7 76.2 499 ripose transport ATP-binding protein 1387 4887 1319005 1319976 972 sp:RBSC_ECOLI Escherichia coli K12 MG1655 45.6 76.9 329 high affinity ribose transport protein rbsC 1388 4888 1320001 1320942 942 sp:RBSB_ECOLI Escherichia coli K12 MG1655 45.9 77.7 305 periplasmic ribose-binding protein rbsB 1389 4889 1320952 1321320 369 sp:RBSD_ECOLI Escherichia coli K12 MG1655 41.7 68.4 139 high affinity ribose transport protein rbsD 1390 4890 1321476 1322111 636 sp:YIW2_YEAST Saccharomyces cerevisiae 31.0 58.0 200 hypothetical protein YIR042c 1391 4891 1322393 1323406 1014 gp:SCF34_13 Streptomyces coelicolor 31.4 60.2 354 iron-siderophore binding lipoprotein SCF34.13c 1392 4892 1323533 1324537 1005 sp:NTCI_RAT Rattus norvegicus (Rat) NTCI 35.8 61.9 268 Na-dependent bile acid transporter 1393 4893 1324778 1326256 1479 gsp:W61467 Staphylococcus aureus WHU 29 43.1 71.8 485 RNA-dependent amidotransferase B ratB 1394 4894 1326378 1327049 672 sp:F4RE_METJA Methanococcus jannaschii 32.6 61.1 172 putative F420-dependent NADH MJ1501 f4re reductase 1395 4895 1330967 1329891 1077 sp:YQJG_ECOLI Escherichia coli K12 yqjG 39.8 66.9 317 hypothetical protein 1396 4896 1331102 1331875 774 pir:A70672 Mycobacterium tuberculosis 39.3 62.4 234 hypothetical protein H37Rv Rv2972c 1397 4897 1331953 1333008 1056 pir:H70855 Mycobacterium tuberculosis 27.4 52.6 325 hypothetical membrane protein H37Rv Rv3005c 1398 4898 1333424 1333188 237 1399 4899 1335280 1333442 1839 gp:AJ012293_1 Corynebacterium glutamicum 99.2 99.4 613 dihydroxy-acid dehydratase ATCC 13032 ilvD 1400 4900 1335975 1335412 564 pir:G70855 Mycobacterium tuberculosis 33.3 68.6 105 hypothetical protein H37Rv Rv3004 1401 4901 1337567 1336095 1473 sp:YILV_CORGL Corynebacterium glutamicum 100.0 100.0 62 hypothetical membrane protein ATCC 13032 yilV 1402 4902 1338609 1338379 231 GP:SSU18930_263 **Sulfolobus** solfataricus 45.0 55.0 66 hypothetical protein 1403 4903 1342072 1342677 606 1404 4904 1342457 1341960 498 sp:NRTD_SYNP7 Synechococcus sp. nrtD 50.9 80.8 167 nitrate transport ATP-binding potein 1405 4905 1342727 1342461 267 sp:MALK_ENTAE Enterobacter aerogenes 46.0 78.2 87 maltose/maltodextrin transport ATP- (Aerobacter aerogenes) malK binding protein 1406 4906 1343675 1342794 882 sp:NRTA_ANASP Anabaena sp strain PCC 7120 28.1 56.8 324 nitrate transporter protein nrtA 1407 4907 1344018 1344464 447 1408 4908 1344440 1344808 369 1409 4909 1344935 1345420 486 sp:DIM6_STRCO Streptomyces coelicolor 39.4 73.2 142 actinorhodin polyketide dimerase 1410 4910 1345486 1346439 954 sp:CZCD_ALCEU Ralstonia eutropha czcD 39.1 72.7 304 cobalt-zinc-cadimium resistance protein 1411 4911 1345487 1345335 153 1412 4912 1346331 1345642 690 1413 4913 1346458 1348272 1815 sp:Y686_METJA Methanococcus jannaschii 22.9 53.7 642 hypothetical protein 1414 4914 1348334 1350076 1743 1415 4915 1350855 1352444 1590 gsp:Y22646 Brevibacterium flavum serA 99.8 100.0 530 D-3-phosphoglycerate dehydrogenase 1416 4916 1352053 1351727 327 SP:YEN1_SCHPO Schizosaccharomyces pombe 29.0 52.0 105 hypothetical serine-rich protein SPAC11G7.01 1417 4917 1352585 1353451 867 1418 4918 1355601 1354540 1062 1419 4919 1355689 1357554 1866 pir:T03476 Rhodobacter capsulatus strain 32.9 63.1 620 hypothetical protein SB1003 1420 4920 1356452 1356853 402 1421 4921 1357557 1358210 654 1422 4922 1358259 1359062 804 sp:HPCE_ECOLI Escherichia coli C hpcE 33.3 59.2 228 homoprotocatechiuate catabolism bifunctional isomerase/decarboxylase [includes: 2-hydroxyhepta-2,4-diene-1,7-dioate isomerase(hhdd isomerase); 5-carboxymethyl-2-oxo-hex-3-ene-1,7- dioate decarboxylase(opet decarboxylase)]

1423 4923 1359052 1359669 618 sp:UBIG_ECOLI Escherichia coli K12 23.4 55.7 192 methyltransferase or 3- demethylubiquinone-9 3-O- methyltransferase 1424 4924 1361295 1360168 1128 sp:DHBC_BACSU Bacillus subtilis dhbC 38.0 70.4 371 isochorismate synthase 1425 4925 1361361 1362848 1488 sp:SYE_BACSU Bacillus subtilis gltX 37.3 69.7 485 glutamyl-tRNA synthetase 1426 4926 1363138 1362926 213 gp:SCJ33_10 Streptomyces coelicolor A3(2) 77.0 90.0 67 transcriptional regulator 1427 4927 1363657 1363142 516 1428 4928 1364253 1363732 522 1429 4929 1364915 1365256 342 1430 4930 1364960 1364340 621 1431 4931 1365180 1364878 303

Detail Description Table CWU - DETL:

sp:RIMM_MYCLE Mycobacterium leprae 52.3 72.1 172 16S rRNA processing protein MLCB250.34. rimM 2250 5750 2164390 2164737 348 pir:B71881 Helicobacter pylori J99 jhp0839 29.0 66.7 69 hpothetical protein 2251 5751 2165309 2164815 495 pir:C47154 Bacillus subtilis 168 rpsP 47.0 79.5 83 30S ribosomal protein S16 2252 5752 2165523 2166098 576 pir:T14151 Mus musculus inv 32.1 61.7 196 inversin 2253 5753 2166990 2166124 867 prf:2512328G Streptococcus agalactiae cylB 26.6 69.1 256 ABC transporter 2254 5754 2167865 2166990 876 prf:2220349C Pyrococcus horikoshii OT3 mtrA 35.5 63.8 318 ABC transporter 2255 5755 2169584 2167944 1641 sp:SR54_BACSU Bacillus subtilis 168 ffh 58.7 78.2 559 signal recognition particle protein 2256 5756 2170426 2171058 633 2257 5757 2171715 2172131 417 2258 5758 2172209 2172877 669 2259 5759 2175288 2173759 1530 sp:FTSY_ECOLI Escherichia coli K12 ftsY 37.0 66.1 505 cell division protein 2260 5760 2176046 2175888 159 2261 5761 2176402 2177103 702 2262 5762 2179502 2176110 3393 sp:AMYH_YEAST Saccharomyces cerevisiae 22.4 46.2 1144 glucan 1,4-alpha-glucosidase or S288C YIR019C sta1 glucoamylase S1/S2 precursor 2263 5763 2180918 2181880 963 2264 5764 2183092 2179628 3465 sp:Y06B_MYCTU Mycobacterium tuberculosis 48.3 72.6 1206 chromosome segregation protein H37Rv Rv2922c smc 2265 5765 2183391 2183110 282 sp:ACYP_MYCTU Mycobacterium tuberculosis 51.1 73.9 92 acylphosphatase H37Rv RV2922.1C 2266 5766 2185258 2183405 1854 2267 5767 2186208 2185351 858 sp:YFER_ECOLI Escherichia coli K12 yfeR 23.9 60.0 305 transcriptional regulator 2268 5768 2186299 2187129 831 pir:S72748 Mycobacterium leprae 39.3 73.5 257 hypothetical membrane protein MLCL581.28c 2269 5769 2187160 2187342 183 2270 5770 2187679 2187233 447 2271 5771 2188306 2187692 615 gp:DNINTREG_3 Dichelobacter nodosus gep 46.8 76.6 188 cation efflux system protein 2272 5772 2189170 2188313 858 sp:FPG_ECOLI Escherichia coli K12 mutM or 36.1 66.7 285 formamidopyrimidine-DNA fpg glycosylase 2273 5773 2189906 2189166 741 pir:B69693 Bacillus subtilis 168 rncS 40.3 76.5 221 ribonuclease III 2274 5774 2190439 2189906 534 sp:Y06F_MYCTU Mycobacterium tuberculosis 35.8 62.5 176 hypothetical protein H37Rv Rv2926c 2275 5775 2191328 2190540 789 sp:Y06G_MYCTU Mycobacterium tuberculosis 50.0 76.9 238 hypothetical protein H37Rv Rv2927c 2276 5776 2191522 2193165 1644 prf:2104260G Streptomyces verticillus 28.3 55.6 559 transport protein 2277 5777 2193165 2194694 1530 sp:CYDC_ECOLI Escherichia coli K12 cydC 26.6 58.8 541 ABC transporter 2278 5778 2196883 2198004 1122 gp:SC9C7_2 Streptomyces coelicolor A3(2) 35.3 62.6 388 hypothetical protein SC9C7.02 2279 5779 2198447 2198007 441 2280 5780 2198475 2199758 1284 pir:A72322 Thermotoga maritima MSB8 21.0 43.7 405 hypothetical protein TM0896 2281 5781 2199808 2201070 1263 sp:HIPO_CAMJE Campylobacter jejuni ATCC 32.9 64.3 353 peptidase 43431 hipO 2282 5782 2201408 2201073 336 pir:S38197 Arabidopsis thaliana SUC1 27.1 51.9 133 sucrose

transport protein 2283 5783 2201584 2201450 135 2284 5784 2201869 2201594 276 2285 5785 2204541 2201992 2550 prf:2513410A Thermococcus litoralis malP 36.1 67.4 814 maltodextrin phosphorylase / glycogen phosphorylase 2286 5786 2205490 2204591 900 sp:YFIE_BACSU Bacillus subtilis 168 yfiE 33.9 66.4 295 hypothetical protein 2287 5787 2208249 2207302 948 sp:LGT_STAAU Staphylococcus aureus FDA 485 31.4 65.5 264 prolipoprotein diacylglyceryl lgt transferase 2288 5788 2209167 2208367 801 sp:TRPG_EMENI Emericella nidulans trpC 29.6 62.1 169 indole-3-glycerol-phosphate synthase/anthranilate synthase component II 2289 5789 2209888 2209232 657 pir:H70556 Mycobacterium tuberculosis 29.4 58.8 228 hypothetical membrane protein H37Rv Rv1610 2290 5790 2210273 2209920 354 sp:HIS3_RHOSH Rhodobacter sphaeroides ATCC 52.8 79.8 89 phosphoribosyl-AMP cyclohydrolase 17023 hisI 2291 5791 2211046 2210273 774 sp:HIS6_CORG Corynebacterium glutamicum 97.3 97.7 258 cyclase AS019 hisF 2292 5792 2211875 2211051 825 prf:2419176B Corynebacterium glutamicum 94.0 94.0 241 inositol monophosphate AS019 impA phosphatase 2293 5793 2212619 2211882 738 gp:AF051846_1 Corynebacterium glutamicum 95.9 97.6 245 phosphoribosylformimino-5- AS019 hisA aminoimidazole carboxamide ribotide isomerase 2294 5794 2213273 2212641 633 gp:AF060558_1 Corynebacterium glutamicum 86.7 92.4 210 glutamine amidotransferase AS019 hisH 2295 5795 2215586 2214321 1266 sp:CMLR_STRLI Streptomyces lividans 66 cmlR 25.6 54.0 402 chloramphenicol resistance protein or transmembrane transport protein 2296 5796 2215863 2215639 225 2297 5797 2216474 2215869 606 sp:HIS7_STRCO Streptomyces coelicolor A3(2) 52.5 81.8 198 imidazoleglycerol-phosphate hisB dehydratase 2298 5798 2217591 2216494 1098 sp:HIS8_STRCO Streptomyces coelicolor A3(2) 57.2 79.3 362 histidinol-phosphate hisC aminotransferase 2299 5799 2218925 2217600 1326 sp:HISX_MYCSM Mycobacterium smegmatis 63.8 85.7 439 histidinol dehydrogenase ATCC 607 hisD 2300 5800 2219159 2220358 1200 gp:SPBC215_13 Schizosaccharomyces pombe 27.2 54.4 342 serine-rich secreted protein SPBC215.13 2301 5801 2221109 2220459 651 2302 5802 2221611 2221919 309 2303 5803 2221828 2221187 642 prf:2321269A Leishmania donovani SAcP-1 29.4 59.7 211 histidine secretory acid phosphatase 2304 5804 2221958 2222518 561 pir:RPECR1 Escherichia coli plasmid RP1 28.9 60.8 204 tet repressor protein tetR 2305 5805 2222528 2225035 2508 prf:2307203B **Sulfolobus acidocaldarius** treX 47.4 75.5 722 glycogen debranching enzyme 2306 5806 2225149 2225949 801 pir:E70572 Mycobacterium tuberculosis 50.0 76.0 258 hypothetical protein H37Rv Rv2622 2307 5807 2226763 2225990 774 gp:SC2G5_27 Streptomyces coelicolor A3(2) 29.9 55.2 268 oxidoreductase SC2G5.27c gip 2308 5808 2227779 2226769 1011 prf:2503399A Sinorhizobium meliloti idhA 35.0 60.9 343 myo-inositol 2-dehydrogenase 2309 5809 2227906 2228901 996 sp:GALR_ECOLI Escherichia coli K12 galR 30.4 64.4 329 galactitol utilization operon repressor 2310 5810 2229896 2229099 798 sp:FHUC_BACSU Bacillus subtilis 168 fhuC 32.9 68.3 246 ferrichrome transport ATP-binding protein or ferrichrome ABC transporter 2311 5811 2230937 2229900 1038 prf:2423441E Vibrio cholerae hutC 36.8 71.1 332 hemin permease 2312 5812 2231294 2230947 348 pir:G70046 Bacillus subtilis 168 yvrC 30.1 68.0 103 iron-binding protein 2313 5813 2231932 2231339 594 pir:G70046 Bacillus subtilis 168 yvrC 34.6 67.6 182 iron-binding protein 2314 5814 2232456 2232016 441 sp:YTFH_ECOLI Escherichia coli K12 ytfH 38.1 73.5 113 hypothetical protein 2315 5815 2232928 2234070 1143 gp:SCI8_12 Streptomyces coelicolor A3(2) 23.4 50.1 355 DNA polymerase III epsilon chain SCI8.12 2316 5816 2234158 2234763 606 2317 5817 2234852 2237284 2433 pir:S65769 Arthrobacter sp. Q36 treY 42.0 68.6 814 maltooligosyl **trehalose** synthase 2318 5818 2237331 2238353 1023 gp:AE002006_4 Deinococcus radiodurans 27.6 52.8 322 hypothetical protein DR1631 2319 5819 2239092

2238694 399 2320 5820 2240042 2239845 198 2321 5821 2240246 2240058 189 2322 5822 2240563 2239508 1056 2323 5823 2240681 2241724 1044 sp:LXA1_PHOLU Photorhabdus luminescens 20.5 54.4 375 alkanal monooxygenase alpha chain ATCC 29999 luxA 2324 5824 2242115 2241738 378 gp:SC7H2_5 Streptomyces coelicolor A3(2) 58.3 79.2 120 hypothetical protein SC7H2.05 2325 5825 2242359 2242129 231 2326 5826 2243035 2244819 1785 pir:S65770 Arthrobacter sp. Q36 treZ 46.3 72.4 568 maltooligosyltrehalose trehalohydrolase 2327 5827 2243043 2242393 651 sp:YVYE_BACSU Bacillus subtilis 168 36.5 72.4 214 hypothetical protein 2328 5828 2246171 2244864 1308 sp:THD1_CORGL Corynebacterium glutamicum 99.3 99.3 436 threonine dehydratase ATCC 13032 ilvA 2329 5829 2246386 2246892 507 2330 5830 2246450 2246295 156 2331 5831 2248208 2247006 1203 pir:S57636 Catharanthus roseus metE 22.7 49.6 415 Corynebacterium glutamicumASO19 2332 5832 2251939 2248358 3582 prf:2508371A Streptomyces coelicolor A3(2) 53.3 80.5 1183 DNA polymerase III dnaE 2333 5833 2252017 2252856 840 sp:RARD_ECOLI Escherichia coli K12 rarD 37.6 73.8 279 chloramphenicol sensitive protein 2334 5834 2253192 2253659 468 sp:HISJ_CAMJE Campylobacter jejuni DZ72 hisJ 21.5 55.7 149 histidine-binding protein precursor 2335 5835 2253725 2254642 918 pir:D69548 Archaeoglobus fulgidus AF2388 22.7 64.7 198 hypothetical membrane protein 2336 5836 2255558 2254683 876 sp:GS39_BACSU Bacillus subtilis 168 ydaD 48.2 80.0 280 short chain dehydrogenase or general stress protein 2337 5837 2257024 2255738 1287 sp:DCDA_PSEAE Pseudomonas aeruginosa lysA 22.9 47.6 445 diaminopimelate (DAP) decarboxylase 2338 5838 2259312 2258362 951 sp:CYSM_ALCEU Alcaligenes eutrophus CH34 32.8 64.3 314 cysteine synthase cysM 2339 5839 2259999 2259421 579 2340 5840 2260931 2260002 930 sp:RLUD_ECOLI Escherichia coli K12 rluD 36.5 61.0 326 ribosomal large subunit pseudouridine synthase D 2341 5841 2261467 2260934 534 sp:LSPA_PSEFL Pseudomonas fluorescens NCIB 33.8 61.7 154 lipoprotein signal peptidase 10586 lspA 2342 5842 2261688 2262689 1002

Detail Description Table CWU - DETL:

H37Rv Rv2345 2496 5996 2410264 2409779 486 gp:AE003565_26 Drosophila melanogaster 24.6 54.4 138 hypothetical protein CG10592 2497 5997 2410861 2410280 582 2498 5998 2412338 2410956 1383 pir:S58522 Thermus aquaticus HB8 46.1 69.9 508 glycyl-tRNA synthetase 2499 5999 2412580 2412948 369 pir:E70585 Mycobacterium tuberculosis 49.4 73.0 89 bacterial regulatory protein, arsR H37Rv Rv2358 furB family 2500 6000 2412992 2413423 432 sp:FUR_ECOLI Escherichia coli K12 fur 34.9 70.5 132 ferric uptake regulation protein 2501 6001 2413568 2415118 1551 pir:A70539 Mycobacterium tuberculosis 24.8 46.7 529 hypothetical protein (conserved in H37Rv Rv1128c C.glutamicum?) 2502 6002 2416089 2415298 792 gp:AF162938_1 Streptomyces coelicolor A3(2) 40.6 67.0 224 hypothetical membrane protein h3u 2503 6003 2417099 2416371 729 sp:UPPS_MICLU Micrococcus luteus B-P 26 uppS 43.4 71.2 233 undecaprenyl diphosphate synthase 2504 6004 2417947 2417222 726 pir:A70586 Mycobacterium tuberculosis 45.7 74.3 245 hypothetical protein H37Rv Rv2362c 2505 6005 2418883 2417969 915 gp:AF072811_1 Streptococcus pneumoniae era 39.5 70.3 296 Era-like GTP-binding protein 2506 6006 2420309 2418990 1320 sp:Y1DE_MYCTU Mycobacterium tuberculosis 52.8 82.4 432 hypothetical membrane protein H37Rv Rv2366 2507 6007 2420900 2420313 588 sp:YN67_MYCTU Mycobacterium tuberculosis 65.0 86.0 157 hypothetical protein H37Rv Rv2367c 2508 6008 2420973 2421236 264 GSP:Y75650 Neisseria meningitidis 45.0 50.0 85 Neisserial polypeptides predicted to be useful antigens for vaccines and diagnostics 2509 6009

2421949 2420900 1050 sp:PHOL_MYCTU Mycobacterium tuberculosis 61.1 84.6 344 phosphate starvation inducible H37Rv Rv2368c phoH protein 2510 6010 2422697 2421975 723 gp:SCC77_19 Streptomyces coelicolor A3(2) 44.0 75.4 248 hypothetical protein SCC77.19c. 2511 6011 2422850 2423791 942 2512 6012 2423845 2422700 1146 prf:2421342B Streptomyces albus dnaJ2 47.1 77.4 380 heat shock protein dnaJ 2513 6013 2424937 2423915 1023 prf:2421342A Streptomyces albus hrcA 48.2 79.6 334 heat-inducible transcriptional repressor (groEL repressor) 2514 6014 2425954 2424965 990 prf:2318256A Bacillus stearothermophilus 33.1 64.1 320 oxygen-independent hemN coproporphyrinogen III oxidase 2515 6015 2426181 2426699 519 sp:AGA1_YEAST Saccharomyces cerevisiae 36.6 64.9 134 agglutinin attachment subunit YNR044WAGA1 precursor 2516 6016 2427468 2426776 693 2517 6017 2428184 2427807 378 2518 6018 2430028 2428184 1845 gp:SC6G10_4 Streptomyces coelicolor A3(2) 48.0 75.1 611 long-chain-fatty-acid-CoA ligase SC6G10.04 2519 6019 2430296 2432413 2118 sp:MALQ_ECOLI Escherichia coli K12 malQ 28.3 55.4 738 4-alpha-glucanotransferase 2520 6020 2432508 2434370 1863 gp:AB005752_1 Lactobacillus brevis plasmid 29.5 64.4 604 ABC transporter, Hop-Resistance horA protein 2521 6021 2433868 2433614 255 GSP:Y74827 Neisseria gonorrhoeae 44.0 51.0 68 Neisserial polypeptides predicted to be useful antigens for vaccines and diagnostics 2522 6022 2434207 2433875 333 GSP:Y74829 Neisseria meningitidis 47.0 53.0 107 polypeptides predicted to be useful antigens for vaccines and diagnostics 2523 6023 2434619 2434440 180 2524 6024 2434776 2434573 204 2525 6025 2436838 2434805 2034 sp:DCP_SALTY Salmonella typhimurium dcp 40.3 68.3 690 peptidyl-dipeptidase 2526 6026 2436871 2438049 1179 gp:AF064523_1 Anisopteromalus calandrae 24.1 45.7 453 carboxylesterase 2527 6027 2438113 2439906 1794 pir:G70983 Mycobacterium tuberculosis 65.2 84.9 594 glycosyl hydrolase or **trehalose** H37Rv Rv0126 synthase 2528 6028 2439906 2440994 1089 pir:H70983 Mycobacterium tuberculosis 32.1 58.8 449 hypothetical protein H37Rv Rv0127 2529 6029 2441589 2441005 585 pir.T07979 Chlamydomonas reinhardtii ipi1 31.8 57.7 189 isopentenyl-diphosphate Delta- isomerase 2530 6030 2441669 2441890 222 2531 6031 2442355 2442792 438 2532 6032 2443356 2441602 1755 2533 6033 2444015 2443356 660 2534 6034 2444551 2444033 519 2535 6035 2444735 2445709 975 gp:CORCSLYS_1 Corynebacterium glutamicum 99.4 100.0 325 beta C-S lyase (degradation of ATCC 13032 aecD aminoethylcysteine) 2536 6036 2445716 2446993 1278 sp:BRNQ_CORGL Corynebacterium glutamicum 99.8 100.0 426 branched-chain amino acid transport ATCC 13032 brnQ system carrier protein (isoleucine uptake) 2537 6037 2447021 2447998 978 sp:LUXA_VIBHA Vibrio harveyi luxA 21.6 49.0 343 alkanal monooxygenase alpha chain 2538 6038 2450844 2450323 522 2539 6039 2451785 2450859 927 gp:AF155772_2 SinoRhizobium meliloti mdcF 25.9 60.5 324 malonate transporter 2540 6040 2454637 2451794 2844 sp:GLCD_ECOLI Escherichia coli K12 glcD 27.7 55.1 483 glycolate oxidase subunit 2541 6041 2454725 2455435 711 sp:YDFH_ECOLI Escherichia coli K12 ydfH 25.6 65.0 203 transcriptional regulator 2542 6042 2455733 2455452 282 2543 6043 2457066 2455720 1347 sp:YGIK_SALTY Salmonella typhimurium ygiK 22.5 57.6 467 hypothetical protein 2544 6044 2457759 2457337 423 2545 6045 2457863 2459371 1509 sp:HBPA_HAEIN Haemophilus influenzae Rd 27.5 55.5 546 heme-binding protein A precursor HI0853 hbpA (hemin-binding lipoprotein) 2546 6046 2459371 2460336 966 sp:APPB_BACSU Bacillus subtilis 168 appB 40.0 73.3 315 oligopeptide ABC transporter (permease) 2547 6047 2460340 2461167 828 sp:DPPC_ECOLI Escherichia coli K12 dppC 43.2 74.5 271 dipeptide transport system permease protein 2548 6048 2461163 2462599 1437 prf:2306258MR Escherichia coli K12 oppD 37.4 66.4 372 oligopeptide transport ATP-binding protein 2549 6049 2462049 2461543 507 PIR:G72536 Aeropyrum pernix

K1 APE1580 35.0 44.0 106 hypothetical protein 2550 6050 2463150 2462602 549 pir:D70367 Aquifex aeolicus VF5 aq_768 29.3 58.0 157 hypothetical protein 2551 6051 2463241 2464143 903 prf:2514301A Rhizobium etli rbsK 41.0 65.0 300 ribose kinase 2552 6052 2464344 2465768 1425 gp:SCM2_16 Streptomyces coelicolor A3(2) 39.9 64.6 466 hypothetical membrane protein SCM2.16c 2553 6053 2465767 2465465 303 2554 6054 2467009 2466038 972 sp:NTCI_HUMAN Homo sapiens 31.3 61.6 284 sodium-dependent transporter or odium Bile acid symporter family 2555 6055 2467077 2467922 846 gp:AF195243_1 Chlamydomonas reinhardtii 28.5 51.2 295 apospory-associated protein C 2556 6056 2470313 2470678 366 2557 6057 2472250 2472819 570 sp:THIX_CORGL Corynebacterium glutamicum 100.0 100.0 133 thiamine biosynthesis protein X ATCC 13032 thiX 2558 6058 2473480 2472893 588 sp:VG66_BPMD Mycobacteriophage D29 66 42.6 65.5 197 hypothetical protein 2559 6059 2473653 2475542 1890 sp:BETP_CORGL Corynebacterium glutamicum 39.8 71.7 601 glycine betaine transporter ATCC 13032 betP 2560 6060 2476497 2477492 996 2561 6061 2477644 2479251 1608 2562 6062 2479379 2479762 384

Detail Description Table CWU - DETL:

624 gp:AF071885_2 Streptomyces coelicolor M145 69.5 88.3 197 ATP-dependent Clp protease clpP2 proteolytic subunit 2 2648 6148 2556580 2555978 603 gp:AF071885_1 Streptomyces coelicolor M145 62.1 85.9 198 ATP-dependent Clp protease clpP1 proteolytic subunit 1 2649 6149 2556599 2556748 150 gp:SIS243537_4 **Sulfolobus** islandicus ORF154 42.9 71.4 42 hypothetical protein 2650 6150 2558106 2556760 1347 sp:TIG_BACSU Bacillus subtilis 168 tig 32.1 66.4 417 trigger factor (prolyl isomerase) (chaperone protein) 2651 6151 2558609 2559103 495 gp:SCD25_17 Streptomyces coelicolor A3(2) 32.5 63.1 160 hypothetical protein SCD25.17 2652 6152 2559157 2560131 975 sp:PBP4_NOCLA Nocardia lactamdurans LC411 25.3 50.9 336 penicillin-binding protein pbp 2653 6153 2560131 2560586 456 prf:2301342A Mus musculus Moa1 27.8 58.3 115 hypothetical protein 2654 6154 2561115 2561363 249 2655 6155 2561920 2561483 438 prf:2513302C Corynebacterium striatum ORF1 54.2 73.2 142 transposase 2656 6156 2562093 2562242 150 2657 6157 2562115 2561990 126 prf:2513302C Corynebacterium striatum ORF1 57.1 82.9 35 hypothetical protein 2658 6158 2562341 2562078 264 prf:2513302C Corynebacterium striatum ORF1 50.7 78.7 75 transposase 2659 6159 2562776 2562387 390 2660 6160 2562963 2563847 885 2661 6161 2564402 2563932 471 sp:LACB_STAAU Staphylococcus aureus NCTC 40.0 71.4 140 galactose-6-phosphate isomerase 8325-4 lacB 2662 6162 2565245 2564550 696 sp:YAMY_BACAD Bacillus acidopullulyticus ORF2 26.2 58.1 248 hypothetical protein 2663 6163 2566231 2565623 609 pir:A70866 Mycobacterium tuberculosis 56.8 80.9 199 hypothetical protein H37Rv Rv2466c 2664 6164 2566345 2568945 2601 sp:AMPN_STRLI Streptomyces lividans pepN 47.5 70.5 890 aminopeptidase N 2665 6165 2569211 2570293 1083 pir:B70206 Borrelia burgdorferi BB0852 25.1 58.1 358 hypothetical protein 2666 6166 2571460 2570309 1152 2667 6167 2571510 2572175 666 2668 6168 2572193 2572348 156 2669 6169 2572677 2572351 327 gp:AF139916_3 Brevibacterium linens ATCC 61.5 81.7 104 phytoene desaturase 9175 crtI 2670 6170 2572977 2572807 171 2671 6171 2573770 2573393 378 2672 6172 2573864 2572659 1206 sp:CRTJ_MYXXA Myxococcus xanthus DK1050 31.2 63.8 381 phytoene dehydrogenase carA2 2673 6173 2574718 2573843 876 sp:CRTB_STRGR Streptomyces griseus JA3933 31.4 58.6 290 phytoene synthase crtB 2674 6174 2575898 2574780 1119 gp:LMAJ9627_3 Listeria monocytogenes IltB 25.8 47.7 392 multidrug resistance transporter 2675 6175 2577213 2575981 1233

2676 6176 2578872 2577232 1641 gp:SYOATPBP_2 Synechococcus elongatus 41.3 71.6 538 ABC transporter ATP-binding protein 2677 6177 2579760 2578879 882 sp:DPPC_BACFI Bacillus firmus OF4 dppC 38.8 73.8 286 dipeptide transport system permease protein 2678 6178 2580707 2579769 939 pir:S47696 Escherichia coli K12 nikB 33.2 62.0 316 nickel transport system permease protein 2679 6179 2582417 2580711 1707 2680 6180 2582564 2584504 1941 2681 6181 2584613 2585926 1314 sp:ARGD_CORGL Corynebacterium glutamicum 31.4 63.5 411 acetylornithine aminotransferase ATCC 13032 argD 2682 6182 2586180 2587763 1584 pir:A70539 Mycobacterium tuberculosis 25.1 47.9 482 hypothetical protein H37Rv Rv1128c 2683 6183 2587976 2588722 747 sp:YA26_MYCTU Mycobacterium tuberculosis 49.1 79.4 218 hypothetical membrane protein H37Rv Rv0364 2684 6184 2589432 2588725 708 sp:PHBB_CHRVI Chromatium vinosum D phbB 28.1 60.0 235 acetoacetyl CoA reductase 2685 6185 2589565 2590302 738 pir:A40046 Streptomyces coelicolor actII 26.7 55.0 240 transcriptional regulator, TetR family 2686 6186 2590697 2591137 441 GSP:Y74375 Neisseria meningitidis 38.0 47.0 94 polypeptides predicted to be useful antigens for vaccines and diagnostics 2687 6187 2592365 2591574 792 gp:AF106002_1 Pseudomonas putida GM73 31.1 65.1 238 ABC transporter ATP-binding protein ttg2A 2688 6188 2592402 2592794 393 gp:MLCB1610_9 Mycobacterium leprae 53.2 77.0 126 globin MLCB1610.14c 2689 6189 2592838 2593965 1128 sp:CHRA_PSEAE Pseudomonas aeruginosa 27.3 60.4 396 chromate transport protein Plasmid pUM505 chrA 2690 6190 2594594 2593968 627 pir:A70867 Mycobacterium tuberculosis 37.8 68.9 196 hypothetical protein H37Rv Rv2474c 2691 6191 2595061 2594597 465 gp:SC6D10_19 Streptomyces coelicolor A3(2) 36.2 61.4 127 hypothetical protein SC6D10.19c 2692 6192 2595808 2595188 621 2693 6193 2595983 2595822 162 pir:B72589 Aeropyrum pernix K1 APE1182 36.4 60.0 55 hypothetical protein 2694 6194 2597715 2596048 1668 sp:YJJK_ECOLI Escherichia coli K12 yjjK 52.8 79.6 563 ABC transporter ATP-binding protein 2695 6195 2598483 2597869 615 pir:E70867 Mycobacterium tuberculosis 31.4 62.2 172 hypothetical protein H37Rv Rv2478c 2696 6196 2600764 2598662 2103 sp:Y05L_MYCLE Mycobacterium leprae 0659 28.0 56.7 700 hypothetical membrane protein 2697 6197 2601461 2602879 1419 pir:C69676 Bacillus subtilis phoB 28.0 52.6 536 alkaline phosphatase 2698 6198 2604573 2605502 930 2699 6199 2604583 2603945 639 2700 6200 2605520 2604609 912 sp:MSMG_STRMU Streptococcus mutans 39.1 76.3 279 multiple sugar-binding transport INGBRITTmsmG system permease protein 2701 6201 2606369 2605527 843 sp:MSMF_STRMU Streptococcus mutans 27.4 67.5 292 multiple sugar-binding transport INGBRITTmsmF system permease protein 2702 6202 2606444 2608117 1674 2703 6203 2607889 2606561 1329 prf:2206392C Thermoanaerobacterium 28.8 63.2 462 maltose-binding protein thermosul amyE 2704 6204 2609426 2608185 1242 2705 6205 2610639 2609512 1128 prf:2308356A Streptomyces reticuli msiK 59.1 79.8 386 ABC transporter ATP-binding protein (ABC-type sugar transport protein) or cellobiose/maltose transport protein 2706 6206 2611523 2612272 750 2707 6207 2611531 2610848 684 prf:2317468A Schizosaccharomyces pombe 37.7 72.7 154 dolichol phosphate mannose dpm1 synthase 2708 6208 2612462 2613151 690 2709 6209 2613712 2614500 789 prf:2516398E Rhodococcus rhodochrous 67.2 89.4 207 aldehyde dehydrogenase plasmid pRTL1 orf5 2710 6210 2614649 2615410 762 prf:2513418A Synechococcus sp. PCC7942 48.6 73.8 183 circadian phase modifier cpmA 2711 6211 2615451 2615795 345 2712 6212 2617120 2615939 1182 pir:A72312 Thermotoga maritima MSB8 35.0 64.6 412 hypothetical membrane protein TM0964 2713 6213 2617246 2617995 750 sp:GIP_ECOLI Escherichia coli K12 gip 41.2 69.4 255 glyoxylate-induced protein 2714 6214 2618072 2618869 798 pir:E70761 Mycobacterium tuberculosis 40.0 57.0 258 ketoacyl reductase H37Rv Rv1544 2715

6215 2618882 2619538 657 sp:ORN_ECOLI Escherichia coli K12 orn 48.0 78.8 179 oligoribonuclease 2716 6216 2620728 2619541 1188 prf:2409378A Salmonella enterica iroD 26.0 50.9 454 ferric enterochelin esterase 2717 6217 2622181 2620973 1209

Detail Description Table CWU - DETL:

protein 2811 6311 2713702 2713842 141 PIR:F81737 Chlamydia muridarum Nigg 71.0 75.0 42 hypothetical protein TC0129 2812 6312 2718187 2717993 195 2813 6313 2719689 2718436 1254 sp:MURA_ACICA Acinetobacter calcoaceticus 44.8 75.3 417 UDP-N-acetylglucosamine 1- NCIB 8250 murA carboxyvinyltransferase 2814 6314 2719750 2720319 570 sp:Y02Y_MYCTU Mycobacterium tuberculosis 66.3 84.2 190 hypothetical protein H37Rv Rv1314c 2815 6315 2721227 2720385 843 gp:SC2G5_15 Streptomyces coelicolor A3(2) 45.9 69.0 281 transcriptional regulator SC2G5.15c 2816 6316 2721702 2721295 408 2817 6317 2721934 2722857 924 sp:CYSK_BACSU Bacillus subtilis 168 cysK 57.1 84.6 305 cysteine synthase 2818 6318 2723064 2723609 546 prf:2417357C Azotobacter vinelandii cysE2 61.1 79.7 172 O-acetylserine synthase 2819 6319 2724057 2723770 288 gp:AE002024_10 Deinococcus radiodurans R1 36.1 65.1 83 hypothetical protein DR1844 2820 6320 2725359 2724478 882 sp:SUCD_COXBU Coxiella burnetii Nine Mile Ph I 52.9 79.4 291 succinyl-CoA synthetase alpha sucD chain 2821 6321 2725619 2725843 225 PIR:F72706 Aeropyrum pernix K1 APE1069 42.0 43.0 75 hypothetical protein 2822 6322 2726577 2725384 1194 sp:SUCC_BACSU Bacillus subtilis 168 sucC 39.8 73.0 400 succinyl-CoA synthetase beta chain 2823 6323 2727145 2726786 360 2824 6324 2728133 2727399 735 gp:AF058302_5 Streptomyces roseofulvus frnE 38.5 71.8 213 frenolicin gene E product 2825 6325 2729025 2728207 819 2826 6326 2730916 2729378 1539 sp:CAT1_CLOKL Clostridium kluyveri cat1 cat1 47.9 77.8 501 succinyl-CoA coenzyme A transferase 2827 6327 2731376 2732518 1143 sp:NIR3_AZOBR Azospirillum brasilense ATCC 38.6 68.5 321 transcriptional regulator 29145 ntrC 2828 6328 2732230 2731424 807 2829 6329 2732636 2733367 732 pir:E70810 Mycobacterium tuberculosis 46.5 81.7 213 phosphate transport system H37Rv Rv0821c phoY-2 regulatory protein 2830 6330 2734351 2733455 897 pir:S68595 Pseudomonas aeruginosa pstB 58.8 82.8 255 phosphate-specific transport component 2831 6331 2735184 2734264 921 gp:MTPSTA1_1 Mycobacterium tuberculosis 51.4 82.2 292 phosphate ABC transport system H37Rv Rv0830 pstA1 permease protein 2832 6332 2736215 2735202 1014 pir:A70584 Mycobacterium tuberculosis 50.2 78.5 325 phosphate ABC transport system H37Rv Rv0829 pstC2 permease protein 2833 6333 2737538 2736414 1125 pir:H70583 Mycobacterium tuberculosis 40.0 56.0 369 phosphate-binding protein S-3 H37Rv phoS2 precursor 2834 6334 2738711 2737836 876 gp:SCD84_18 Streptomyces coelicolor A3(2) 34.3 60.0 315 acetyltransferase SCD84.18c 2835 6335 2738771 2739553 783 2836 6336 2740650 2739556 1095 sp:BMRU_BACSU Bacillus subtilis 168 bmrU 24.7 55.2 344 hypothetical protein 2837 6337 2740670 2741356 687 pir:E70809 Mycobacterium tuberculosis 44.9 74.2 225 hypothetical protein H37Rv Rv0813c 2838 6338 2742577 2741636 942 gp:AF193846_1 Solanum tuberosum BCAT2 28.6 56.0 259 branched-chain amino acid aminotransferase 2839 6339 2742685 2743785 1101 gp:AB003158_6 Corynebacterium 58.5 79.0 352 hypothetical protein ammoniagenes ATCC 6872 ORF4 2840 6340 2744010 2744222 213 pir:B70809 Mycobacterium tuberculosis 58.6 81.0 58 hypothetical protein H37Rv Rv0810c 2841 6341 2745954 2744881 1074 gp:AB003158_5 Corynebacterium 81.0 94.2 347 5'-phosphoribosyl-5-aminoimidazole ammoniagenes ATCC 6872 synthetase purM 2842 6342 2747564 2746083 1482 gp:AB003158_4 Corynebacterium 70.3 89.0 482

amidophosphoribosyl transferase ammoniagenes ATCC 6872 purF 2843 6343 2748057 2747683 375 pir:H70536 Mycobacterium tuberculosis 57.3 75.8 124 hypothetical protein H37Rv Rv0807 2844 6344 2748095 2749111 1017 gp:AB003158_2 Corynebacterium 75.9 94.0 315 hypothetical protein ammoniagenes ATCC 6872 ORF2 2845 6345 2749902 2749162 741 gp:AB003158_1 Corynebacterium 67.7 87.1 217 hypothetical membrane protein ammoniagenes ATCC 6872 ORF1 2846 6346 2751918 2752103 186 GP:SSU18930_21 **Sulfolobus** solfataricus 64.0 71.0 42 hypothetical protein 4 2847 6347 2752312 2750027 2286 gp:AB003162_3 Corynebacterium 77.6 89.5 763 5'-phosphoribosyl-N- ammoniagenes ATCC 6872 formylglycinamidine synthetase purL 2848 6348 2752402 2753121 720 2849 6349 2752995 2752327 669 gp:AB003162_2 Corynebacterium 80.3 93.3 223 5'-phosphoribosyl-N- ammoniagenes ATCC 6872 formylglycinamidine synthetase purQ 2850 6350 2753237 2752995 243 gp:AB003162_1 Corynebacterium 81.0 93.7 79 hypothetical protein ammoniagenes ATCC 6872 purorf 2851 6351 2753298 2753819 522 2852 6352 2753804 2753328 477 prf:2420329A Lactococcus lactis gpo 46.2 77.9 158 gluthatione peroxidase 2853 6353 2753992 2756739 2748 prf:2216389A Aeromonas hydrophila JMP636 28.0 51.5 965 extracellular nuclease nucH 2854 6354 2756851 2757126 276 2855 6355 2757815 2757129 687 pir:C70709 Mycobacterium tuberculosis 37.4 68.7 211 hypothetical protein H37Rv Rv0784 2856 6356 2759200 2757863 1338 sp:DCTA_SALTY Salmonella typhimurium LT2 49.0 81.6 414 C4-dicarboxylate transporter dctA 2857 6357 2761649 2759532 2118 prf:2408266A Pseudomonas sp. WO24 dapb1 41.8 70.6 697 dipeptidyl aminopeptidase 2858 6358 2762452 2761829 624 2859 6359 2762675 2761785 891 gp:AB003161_3 Corynebacterium 70.1 89.1 294 5'-phosphoribosyl-4-N- ammoniagenes ATCC 6872 succinocarboxamide-5-amino purC imidazole synthetase 2860 6360 2764931 2763504 1428 gp:AB003161_2 Corynebacterium 85.3 95.0 477 adenylosuccino lyase ammoniagenes ATCC 6872 purB 2861 6361 2766135 2764978 1158 sp:AAT_SULSO **Sulfolobus** solfataricus ATCC 28.1 62.3 395 aspartate aminotransferase 49255 2862 6362 2767420 2766158 1263 gp:AB003161_1 Corynebacterium 71.1 86.4 425 5'-phosphoribosylglycinamide ammoniagenes ATCC 6872 synthetase purD 2863 6363 2767580 2767993 414 sp:YHIT_MYCLE Mycobacterium leprae u296a 53.7 80.2 136 histidine triad (HIT) family protein 2864 6364 2768137 2767703 435 2865 6365 2769095 2768343 753 pir:S62195 Methanosarcina barkeri orf3 26.8 56.4 243 hypothetical protein 2866 6366 2770511 2769156 1356 sp:DTPT_LACLA Lactococcus lactis subsp. lactis 30.1 67.6 469 di-/tripeptide transpoter dipT 2867 6367 2770714 2771982 1269 sp:BIOA_CORGL Corynebacterium glutamicum 95.7 98.8 423 adenosylmethionine-8-amino-7- (Brevibacterium flavum) MJ233 oxononanoate aminotransferase or bioA 7,8-diaminopelargonic acid aminotransferase 2868 6368 2771989 2772660 672 sp:BIOD_CORGL Corynebacterium glutamicum 98.7 99.6 224 dethiobiotin synthetase (Brevibacterium flavum) MJ233 bioD 2869 6369 2774098 2772644 1455 gp:AF049873_3 Lactococcus lactis M71plasmid 31.3 70.5 335 two-component system sensor pND306 histidine kinase 2870 6370

Detail Description Table CWU - DETL:

2774814 2774110 705 prf:2222216A Thermotoga maritima drrA 42.0 72.7 231 two-component system regulatory protein 2871 6371 2775689 2774937 753 sp:TIPA_STRLI Streptomyces lividans tipA 37.4 69.5 249 transcriptional activator 2872 6372 2776879 2775740 1140 prf:2419350A Arthrobacter sp. DK-38 30.9 53.9 382 metal-activated pyridoxal enzyme or low specificity D-Thr aldolase 2873 6373 2778504 2776768 1737 gp:ECOPOXB8G_1 Escherichia coli K12

poxB 46.3 75.8 574 pyruvate oxidase 2874 6374 2778965 2780446 1482 prf.2212334B Staphylococcus aureus plasmid 33.3 68.9 504 multidrug efflux protein pSK23 qacB 2875 6375 2780439 2780969 531 sp:YCDC_ECOLI Escherichia coli K12 ycdC 30.4 68.5 92 transcriptional regulator 2876 6376 2780996 2782315 1320 pir:D70551 Mycobacterium tuberculosis 45.6 78.4 421 hypothetical membrane protein H37Rv Rv2508c 2877 6377 2784481 2782340 2142 2878 6378 2785615 2784656 960 gp.AF096929_2 Rhodococcus erythropolis SQ1 34.3 62.1 303 3-ketosteroid dehydrogenase kstD1 2879 6379 2786355 2785651 705 sp.ALSR_BACSU Bacillus subtilis 168 alsR 37.1 69.0 232 transcriptional regulator, LysR family 2880 6380 2787782 2788594 813 pir:C70982 Mycobacterium tuberculosis 28.4 52.9 278 hypothetical protein H37Rv Rv3298c lpqC 2881 6381 2789399 2788587 813 pir:C69862 Bacillus subtilis 168 ykrA 26.7 55.6 288 hypothetical protein 2882 6382 2789935 2789477 459 2883 6383 2790152 2790550 399 pir:A45264 Oryctolagus cuniculus kidney 28.6 50.7 140 hypothetical protein cortex rBAT 2884 6384 2790946 2792448 1503 pir:B70798 Mycobacterium tuberculosis 36.0 64.0 464 hypothetical membrane protein H37Rv Rv3737 2885 6385 2792531 2792857 327 pir:S41307 Streptomyces griseus hrdB 32.3 50.3 155 transcription initiation factor sigma 2886 6386 2792873 2794327 1455 sp:TPS1_SCHPO Schizosaccharomyces pombe 38.8 66.7 487 **trehalose**-6-phosphate synthase tps1 2887 6387 2794300 2794812 513 2888 6388 2794870 2795637 768 sp:OTSB_ECOLI Escherichia coli K12 otsB 27.4 57.6 245 **trehalose**-phosphatase 2889 6389 2796749 2795676 1074 sp:CCPA_BACME Bacillus megaterium ccpA 24.7 60.2 344 glucose-resistance amylase regulator 2890 6390 2796865 2797806 942 sp:ZNUA_HAEIN Haemophilus influenzae Rd 22.4 46.7 353 high-affinity zinc uptake system HI0119 znuA protein 2891 6391 2797820 2798509 690 gp:AF121672_2 Staphylococcus aureus 8325-4 31.4 63.2 223 ABC transporter mreA 2892 6392 2798837 2799391 555 pir:E70507 Mycobacterium tuberculosis 60.0 87.4 135 hypothetical membrane protein H37Rv Rv2060 2893 6393 2799535 2801034 1500 pir:A69426 Archaeoglobus fulgidus 23.4 52.5 303 transposase (ISA0963-5) 2894 6394 2801113 2801313 201 2895 6395 2803246 2801558 1689 gp:AF096929_2 Rhodococcus erythropolis SQ1 32.1 62.0 561 3-ketosteroid dehydrogenase kstD1 2896 6396 2803996 2803250 747 2897 6397 2804691 2804074 618 pir:B72359 Thermotoga maritima MSB8 34.3 56.4 204 lipopolysaccharide biosynthesis bplA protein or oxidoreductase or dehydrogenase 2898 6398 2805110 2804676 435 sp:MI2D_BACSU Bacillus subtilis 168 idh or iolG 35.2 69.5 128 dehydrogenase or myo-inositol 2- dehydrogenase 2899 6399 2805967 2805113 855 sp:SHIA_ECOLI Escherichia coli K12 shiA 30.5 67.5 292 shikimate transport protein 2900 6400 2806441 2806016 426 sp:SHIA_ECOLI Escherichia coli K12 shiA 43.1 80.8 130 shikimate transport protein 2901 6401 2807252 2806599 654 gp:SC5A7_19 Streptomyces coelicolor A3(2) 32.6 55.7 212 transcriptional regulator SC5A7.19c 2902 6402 2808364 2807426 939 sp:PT56_YEAST Saccharomyces cerevisiae 22.8 47.3 334 ribosomal RNA ribose methylase or YOR201C PET56 tRNA/rRNA methyltransferase 2903 6403 2809778 2808399 1380 sp:SYC_ECOLI Escherichia coli K12 cysS 42.2 68.8 464 cysteinyl-tRNA synthetase 2904 6404 2811806 2809824 1983 prf:2511335C Lactococcus lactis sacB 47.0 77.0 668 PTS system, enzyme II sucrose protein (sucrose-specific IIABC component) 2905 6405 2813258 2811960 1299 gp:AF205034_4 Clostridium acetobutylicum 35.3 56.9 473 sucrose 6-phosphate hydrolase or ATCC 824 scrB sucrase 2906 6406 2814037 2813279 759 sp:NAGB_ECOLI Escherichia coli K12 nagB 38.3 69.4 248 glucosamine-6-phosphate isomerase 2907 6407 2815232 2814081 1152 sp:NAGA_VIBFU Vibrio furnissii SR1514 manD 30.2 60.3 368 N-acetylglucosamine-6-phosphate deacetylase 2908 6408 2815458 2816393 936 sp:DAPA_ECOLI Escherichia coli K12 dapA 28.2 62.1 298 dihydrodipicolinate

synthase 2909 6409 2816409 2817317 909 sp:GLK_STRCO Streptomyces coelicolor A3(2) 28.7 57.6 321 glucokinase SC6E10.20c glk 2910 6410 2817363 2818058 696 prf:2516292A Clostridium perfringens NCTC 36.4 68.6 220 N-acetylmannosamine-6-phosphate 8798 nanE epimerase 2911 6411 2818313 2818137 177 2912 6412 2819564 2818350 1215 sp:NANH_MICVI Micromonospora viridifaciens 24.8 50.3 439 sialidase precursor ATCC 31146 nadA 2913 6413 2820285 2819557 729 gp:AF181498_1 Rhizobium etli ansR 26.6 57.2 222 L-asparagine permease operon repressor 2914 6414 2820584 2822191 1608 gp:BFU64514_1 Bacillus firmus OF4 dppA 22.5 51.4 560 dipeptide transporter protein or heme-binding protein 2915 6415 2822387 2823337 951 sp:DPPB_BACFI Bacillus firmus OF4 dappB 31.9 64.3 342 dipeptide transport system permease protein 2916 6416 2824274 2825341 1068 sp:OPPD_BACSU Bacillus subtilis 168 oppD 46.5 78.3 314 oligopeptide transport ATP-binding protein 2917 6417 2825341 2826156 816 sp:OPPF_LACLA Lactococcus lactis oppF 43.4 78.7 258 oligopeptide transport ATP-binding protein 2918 6418 2826835 2826215 621 sp:RHTB_ECOLI Escherichia coli K12 rhtB 28.5 62.7 193 homoserine/homoserin lactone efflux protein or lysE type translocator 2919 6419 2826922 2827404 483 prf:2309303A Bradyrhizobium japonicum lrp 31.0 66.2 142 leucine-responsive regulatory protein 2920 6420 2827817 2827458 360 2921 6421 2828383 2827904 480 pir:C70607 Mycobacterium tuberculosis 55.9 86.2 152 hypothetical protein H37Rv Rv3581c 2922 6422 2829146 2828379 768 sp:Y18T_MYCTU Mycobacterium tuberculosis 46.4 71.5 235 hypothetical protein H37Rv Rv3582c 2923 6423 2829749 2829156 594 pir:H70803 Mycobacterium tuberculosis 73.3 91.1 157 transcription factor H37Rv Rv3583c 2924 6424 2830057 2830779 723 prf:2214304A Mycobacterium tuberculosis 43.5 70.0 223 two-component system response H37Rv Rv3246c mtrA regulator 2925 6425 2830779 2831894 1116 sp:BAES_ECOLI Escherichia coli K12 baeS 29.3 67.7 341 two-component system sensor histidine kinase 2926 6426 2832085 2832666 582 2927 6427 2832790 2834181 1392 sp:RADA_ECOLI Escherichia coli K12 radA 41.5 74.3 463 DNA repair protein RadA 2928 6428 2834188 2835285 1098 sp:YACK_BACSU Bacillus subtilis 168 yacK 40.3 73.3 345 hypothetical protein 2929 6429 2835969 2835283 687 pir:D70804 Mycobacterium tuberculosis 29.4 53.3 231 hypothetical protein H37Rv Rv3587c 2930 6430 2837499 2836048 1452 gp:PPU96338_1 Pseudomonas putida NCIMB 59.5 85.1 471 p-hydroxybenzaldehyde 9866 plasmid pRA4000 dehydrogenase 2931 6431 2837737 2837591 147 2932 6432 2838576 2837956 621 pir:T08204 Chlamydomonas reinhardtii ca1 36.7 66.2 210 mitochondrial carbonate dehydratase beta 2933 6433 2838643 2839521 879 gp:AF121797_1 Streptomyces antibioticus IMRU 48.4 70.7 283 A/G-specific adenine glycosylase 3720 mutY 2934 6434 2839562 2840716 1155 2935 6435 2841063 2840758 306 2936 6436 2841075 2841848 774 gp:AB009078_1 Brevibacterium saccharolyticum 99.2 99.6 258 L-2.3-butanediol dehydrogenase 2937 6437 2842130 2842453 324 2938 6438 2842493 2843233 741 2939 6439 2843405 2843716 312 2940 6440 2843722 2843432 291 pir:E70552 Mycobacterium tuberculosis 48.5 69.1 97 hypothetical protein H37Rv Rv3592 2941 6441 2845139 2845558 420 GSP:Y29188 Pseudomonas aeruginosa 57.0 63.0 99 virulence factor ORF24222 2942 6442 2845889 2846101 213 GSP:Y29193 Pseudomonas aeruginosa 54.0 55.0 72 virulence factor ORF25110 2943 6443 2846186 2846506 321 GSP:Y29193 Pseudomonas aeruginosa 74.0 75.0 55 virulence factor ORF25110 2944 6444 2846940 2844166 2775 sp:MECB_BACSU Bacillus subtilis 168 mecB 58.5 86.2 832 ClpC adenosine triphosphatase/ ATP-binding proteinase 2945 6445 2847229 2848659 1431 gp:AB035643_1 Bacillus cereus ts-4 impdh 37.1 70.2 469 inosine monophosphate dehydrogenase 2946 6446 2848769 2849779 1011 pir:JC6117 Rhodococcus rhodochrous nitR 24.7 62.7 316 transcription factor 2947 6447 2850031 2851815 1785 sp:PH2M_TRICU

Trichosporon cutaneum ATCC 33.5 60.9 680 phenol 2-monooxygenase 46490 2948 6448 2852017 2853732 1716 2949 6449 2853769 2855709 1941 2950 6450 2855795 2857516 1722 2951 6451 2859044 2859205 162 2952 6452 2859055 2857613 1443 gp:AF237667_1 Corynebacterium glutamicum 100.0 100.0 481 lincomycin resistance protein lmrB 2953 6453 2860145 2859195 951 pir:G70807 Mycobacterium tuberculosis 26.7 55.8 240 hypothetical protein H37Rv Rv3517 2954 6454 2862082 2860505 1578 gp:AB012100_1 Bacillus stearothermophilus lysS 41.7 71.2 511 lysyl-tRNA synthetase 2955 6455 2862929 2862132 798 gp:CGPAN_2 Corynebacterium glutamicum 29.9 52.6 268 pantoate-beta-alanine ligase

PGPUB-DOCUMENT-NUMBER: 20020164723

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164723 A1

TITLE: Method of producing saccharide preparations

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liaw, Gin C.	Decatur	IL	US	
Pedersen, Sven	Gentofte		DK	
Hendriksen, Hanne Vang	Holte		DK	
Svendsen, Allan	Birkerod		DK	
Nielsen, Bjarne	Virum		DK	
Ronfeldt	Farum		DK	
Nielsen, Rudy Illum				

APPL-NO: 09/ 908395

DATE FILED: July 18, 2001

RELATED-US-APPL-DATA:

child 09908395 A1 20010718 parent continuation-of 09632392 20000804 US GRANTED parent-patent 6303346 US child 09632392 20000804 US parent continuation-of 09499531 20000210 US GRANTED parent-patent 6136571 US child 09499531 20000210 US parent continuation-of 09198672 19981123 US GRANTED parent-patent 6129788 US child 09198672 19981123 US parent continuation-in-part-of 09107657 19980630 US ABANDONED child 09107657 19980630 US parent continuation-in-part-of 08979673 19971126 US ABANDONED

US-CL-CURRENT: 435/96

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/632,392, filed on Aug. 4, 2000, now allowed, which is a continuation of U.S. patent application Ser. No. 09/499,531, filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. patent application Ser. No. 09/198,672, filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. patent application Ser. No. 09/107,657, filed on Jun. 30, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/979,673, filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference.

[0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, **trehalose,** isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

[0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, **trehalose,** isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

---------- KWIC ---------

Cross Reference to Related Applications Paragraph - CRTX:

[0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, **trehalose,** isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

Cross Reference to Related Applications Paragraph - CRTX:

[0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, **trehalose,** isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent

steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

Summary of Invention Paragraph - BSTX:

[0022] **Trehalose** syrups

Summary of Invention Paragraph - BSTX:

[0023] **Trehalose** (.alpha.-D-glucopyranosyl .alpha.-D-glucopyranoside) is a non reducing disaccharide with two glucose residues bound by a .alpha.-1,1 linkage.

Summary of Invention Paragraph - BSTX:

[0024] Enzymatic processes for producing **trehalose** from starch or maltooligosaccharides are described by, e.g., Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550); Kazuhisa et al. (1997), Starch 49, no. 1. p. 26-30; and in EP 764,720.

Summary of Invention Paragraph - BSTX:

[0039] It has now been found that in a method of producing mono and/or oligosaccharides from starch, including dextrose, **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, the efficiency can be improved significantly, and the costs lowered, if in the saccharification (or hydrolyzing) step, after the liquefaction step, the syrup is subjected to one or more high temperature membrane separation steps, and the saccharification enzyme is returned to the saccharification step. According to the method of the present invention, the membrane separation step may be regarded as an integral part of the saccharification step.

Summary of Invention Paragraph - BSTX:

[0042] When producing saccharides with more than one saccharide unit, i.e., **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides the hydrolyzing step (after the liquefaction step) is followed by an ultra and microfiltration step or a micro and ultrafiltration step.

Summary of Invention Paragraph - BSTX:

[0047] In its second aspect, the invention provides a method for the production of a mono and/or oligosaccharide preparation of, e.g., dextrose, **trehalose,** isomaltooligosaccharides, cyclodextrins and maltooligosaccharides, which method comprises an enzymatic saccharification step, and the subsequent steps of

Detail Description Paragraph - DETX:

[0068] According to the method of the invention, the retentate from the membrane separation is conveyed back (re-circulated) to the saccharification step. Preferably the retentate from the membrane separation is re-circulated to a saccharification stage in the saccharification step, at which stage the content of the reaction mixture matches the content of the retentate with respect to the saccharide, such as glucose, **trehalose,** isomaltooligosaccharide, cyclodextrin or maltooligosaccharide.

Detail Description Paragraph - DETX:

[0073] When producing **trehalose,** the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% **trehalose**.

Detail Description Paragraph - DETX:

[0076] In the context of the present invention a membrane separation step comprises a microfiltration step followed by an ultrafiltration step or an ultrafiltration step followed by a microfiltration step when producing **trehalose,** isomaltooligosaccharides, cyclodextrins and malto-oligosaccharides.

Detail Description Paragraph - DETX:

[0079] In a preferred embodiment, the membrane separation steps comprises a microfiltration step and an ultrafiltration step, applied in the order specified. This embodiment is particularly useful for the production of a syrup holding from about 95 to about 96% glucose, or from 10-40% isomaltose, or 30 to above 80% maltose, or 75-90% **trehalose,** or 30-60% cyclodextrins.

Detail Description Paragraph - DETX:

[0102] Production of **trehalose** (containing 75-90% **trehalose**)

Detail Description Paragraph - DETX:

[0103] In the saccharification step, when producing **trehalose,** liquefied starch is subjected to the action of an enzyme capable of first converting maltooligosaccharide (from the liquefaction step) into the non reducing saccharide maltooligosyl **trehalose** by intramolecular transglycosylation followed by a subsequent step of hydrolyzing the reaction product of the first step (i.e., maltooligosyl **trehalose) into trehalose**. The saccharification step may be performed using maltooligosyl **trehalose** synthase (MTSase) and maltooligosyl **trehalose** trehalohydrolase (MTHase), e.g., the two enzymes described by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550). MTSase and MTHase act on amylose or starch to produce **trehalose**.

Detail Description Paragraph - DETX:

[0104] Another enzymatic process for producing **trehalose** from starch or maltooligosaccharides (see Kato et al., (1996), Biosci. Biotech. Biochem., 60 (3), p. 546-550) involves using **trehalose**-producing enzymes, a glycosyltransferase and an amylase, respectively, from the hyperthermophilic archae **Sulfolobus** solfataricus KM1.

Detail Description Paragraph - DETX:

[0105] Further, EP 764720 also describes using two enzymes from Solfolobus spp. for preparing **trehalose** from starch or maltooligosacchairdes.

Detail Description Paragraph - DETX:

[0124] When producing **trehalose,** the feed stream subjected to membrane separation originating from the saccharification stage holds of from about 50 to about 90%, preferably of from about 60 to 90%, more preferred of from about 75-90% **trehalose**.

Detail Description Paragraph - DETX:

[0141] A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium **Sulfolobus acidocaldarius** (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as **Sulfolobus acidocaldarius** ATCC33909 and from a strain of Rhodethermus marius.

Detail Description Paragraph - DETX:

[0153] Preferably, the saccharification step, when producing **trehalose** is performed in presence of a MTSase and MTHase, e.g., the enzymes disclosed by Masaru et al. (1996), Biosci. Biotech. Biochem., 60 (3), 546-550).

Claims Text - CLTX:

33. The method according to claim 32, in which the MTSase and MTHase is derived from a strain of **Sulfolobus,** such as S. **Acidocaldarius,** especially S. **acidocaldarius** ATCC 33909.

Claims Text - CLTX:

44. The method according to claim 31, for the production of a **trehalose** preparation holding of from about 75 to about 90% **trehalose**.

Claims Text - CLTX:

79. The method according to claim 78, in which the MTSase and MTHase is derived from a strain of **Sulfolobus,** such as S. **acidocaldarius,** especially S. **acidocaldarius** ATCC 33909.

Claims Text - CLTX:

90. The method according to claim 77, for the production of a **trehalose** preparation holding of from about 75 to about 90% **trehalose**.

PGPUB-DOCUMENT-NUMBER: 20020160402

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020160402 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen Albert	Dallas	TX	US	

APPL-NO: 10/ 077392

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077392 A1 20020215 parent division-of 09535366 20000324 US PENDING
non-provisional-of-provisional 60125864 19990324 US
non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/6,435/320.1 ,435/325 ,435/455 ,435/69.1 ,435/7.1

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0092] In certain embodiments, the organism is an archaea (a.k.a.

archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus,** a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0203] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. Azuma et al., (1988) show that **trehalose** dimycolate administration correlates with augmented resistance to influenza virus infection in mice. **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0208] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with **trehalose** dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0221] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020155508

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155508 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen Albert	Dallas	TX	US	

APPL-NO: 10/ 077247

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077247 A1 20020215 parent division-of 09535366 20000324 US GRANTED parent-patent 6410241 US non-provisional-of-provisional 60125864 19990324 US non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/7.2,435/455 ,435/6 ,435/91.2

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0095] In certain embodiments, the organism is an archaea (a.k.a.

archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus,** a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0206] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. Azuma et al., (1988) show that **trehalose** dimycolate administration correlates with augmented resistance to influenza virus infection in mice. **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0211] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with **trehalose** dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0224] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020151060

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020151060 A1

TITLE: PEI: DNA vector formulations for in vitro and in vivo gene delivery

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cristiano, Richard J.	Pearland	TX	US	
Yamashita, Motoyuki	Kochi City		JP	

APPL-NO: 09/ 962922

DATE FILED: September 25, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60235237 20000925 US
non-provisional-of-provisional 60235635 20000926 US

US-CL-CURRENT: 435/455,424/486 ,514/44

ABSTRACT:

The present invention relates generally to the fields of nucleic acid transfection. More particularly, it concerns novel polycation:nucleic acid compositions, methods of preparation of such compositions and methods of transfecting cells with such compositions.

[0001] This application claims the priority of U.S. Provisional Patent Application Ser. No. 60/235,237, filed Sep. 25, 2000 and U.S. Provisional Patent Application Ser. No. 60/235,635, filed Sep. 26, 2000, both of which disclosures are specifically incorporated herein by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0275] In certain embodiments, the organism is an archaea (a.k.a. archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus,** a metallosphaera, an acidianus, a

thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0482] Another adjuvant contemplated for use in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. **Trehalose** dimycolate administration has been shown to correlate with augmented resistance to influenza virus infection in mice (Azuma et al., 1988). **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0487] One group of adjuvants preferred for use in the invention are the detoxified endotoxins, such as the refined detoxified endotoxin of U.S. Pat. No. 4,866,034. These refined detoxified endotoxins are effective in producing adjuvant responses in mammals. Of course, the detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. For example, combination of detoxified endotoxins with **trehalose** dimycolate is particularly contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

PGPUB-DOCUMENT-NUMBER: 20020150940

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020150940 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen Albert	Dallas	TX	US	

APPL-NO: 10/ 077232

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077232 A1 20020215 parent continuation-of 09535366 20000324 US GRANTED parent-patent 6410241 US non-provisional-of-provisional 60125864 19990324 US non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/6,435/455 ,435/7.1 ,435/91.2

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0092] In certain embodiments, the organism is an archaea (a.k.a.

archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus,** a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0203] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. Azuma et al., (1988) show that **trehalose** dimycolate administration correlates with augmented resistance to influenza virus infection in mice. **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0208] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with **trehalose** dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0221] Adjuvants that may be used include IL-1, IL-2, IL4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020146733

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146733 A1

TITLE: Linear and circular expression elements

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sykes, Kathryn F.	Dallas	TX	US	
Johnston, Stephen Albert	Dallas	TX	US	

APPL-NO: 10/ 077621

DATE FILED: February 15, 2002

RELATED-US-APPL-DATA:

child 10077621 A1 20020215 parent division-of 09535366 20000324 US PENDING
non-provisional-of-provisional 60125864 19990324 US
non-provisional-of-provisional 60127222 19990331 US

US-CL-CURRENT: 435/6,435/455 ,435/69.1 ,435/7.1 ,435/91.2

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999 and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0092] In certain embodiments, the organism is an archaea (a.k.a.

archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus,** a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacteriales, methanomicrobiales, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detail Description Paragraph - DETX:

[0203] A preferred adjuvant in the present invention is BCG. BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. Azuma et al., (1988) show that **trehalose** dimycolate administration correlates with augmented resistance to influenza virus infection in mice. **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detail Description Paragraph - DETX:

[0208] The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with **trehalose** dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detail Description Paragraph - DETX:

[0221] Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

PGPUB-DOCUMENT-NUMBER: 20020120116

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020120116 A1

TITLE: ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES

PUBLICATION-DATE: August 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
KUNSCH, CHARLES A.	ATLANTA	GA	US	
DILLON, PATRICK J.	CARLSBAD	CA	US	
BARASH, STEVEN	ROCKVILLE	MD	US	

APPL-NO: 09/ 070927

DATE FILED: May 4, 1998

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

US-CL-CURRENT: 536/23.2,435/252.3 ,435/320.1 ,435/69.1 ,435/70.1 ,435/71.1 ,530/350 ,530/387.9 ,800/13

ABSTRACT:

The present invention provides polynucleotide sequences of the genome of Enterococcus faecalis, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

[0001] This application claims benefit of 35 U.S.C. section 119(e) based on copending U.S. Provisional Application Serial No. 60/046,655, filed May 16, 1997; 60/044,031, filed May 6, 1997; and 60/066,099, filed Nov. 14, 1997. Provisional Application Serial No. 60/066,099, filed Nov. 14, 1997 is herein incorporated by reference in its entirety.

---------- KWIC ---------

Detail Description Table CWU - DETL:

product [Bacillus subtilis] 68 53 363 8 12517 9950 gi.vertline.1652980
H(+)-transporting ATPase [Synechocystis 68 46 sp.] 368 3 1269 1736

gnl.vertline.PID.vertline.e209005 homologous to ORF2 in nrdEF operons of 68 37
E.coli and S.typhimurium [Lactococcus lactis] 386 11 6564 6115
gi.vertline.765072 ORF3 [Staphylococcus aureus] 68 46 395 3 935 729
gi.vertline.5521 ORF 3 (AA 1-90) [Bacteriophaqe phi-105] 68 34 399 8 6073 6519
gi.vertline.153584 biotin carboxyl carrier protein 68 53 [Streptococcus
mutans] sp.vertline.P29337.vertlin- e.BCCP_STRMU BIOTIN CARBOXYL CARRIER
PROTEIN (BCCP). 408 3 2289 1336 gi.vertline.41572 GlnP (AA 1-219) [Escherichia
coli] 68 40 420 1 559 2 gi.vertline.1592142 ABC transporter, probable
ATP-binding 68 51 subunit [Methanococcus jannaschii] 423 2 254 1294
gi.vertline.1773109 similar to S. typhimurium apbA 68 47 [Escherichia coli]
423 3 1465 2421 gi.vertline.1653032 hypothetical protein [Synechocystis sp.]
68 40 428 1 859 2 gi.vertline.1652454 hypothetical protein [Synechocystis
sp.] 68 48 432 7 4626 3901 gi.vertline.1573285 hypothetical [Haemophilus
influenzae] 68 55 434 1 90 1889 gi.vertline.1542975 AbcB
[Thermoanaerobacterium 68 50 thermosulfurigenes] 441 5 4674 5156
gi.vertline.467437 unknown [Bacillus subtilis] 68 48 455 4 3835 4080
gi.vertline.19815 luminal binding protein (BiP) [Nicotiana 68 40 tabacum]
530 2 394 546 gi.vertline.763326 unknown [Saccharomnyces cerevisiae] 68 42
531 2 810 622 gi.vertline.1146183 putative [Bacillus subtilis] 68 51 537 3
1353 1192 gi.vertline.929968 ORFA; similar to B. anthracis WeyAR 68 56
element ORFA; putative ransposase [Bacillus anthracis] 539 3 2725 2231
gi.vertline.1353537 dUTPase [Bacteriophage rlt] 68 53 569 1 3 446
gi.vertline.146544 18 kD protein [Eschenichia coli] 68 47 591 2 656 174
gi.vertline.1039479 ORFU [Lactococcus lactis] 68 42 652 2 739 1032
gi.vertline.1303715 YrkP [Bacillus subtilis] 68 50 671 2 436 1617
gi.vertline.413959 ipa-35d galK gene product [Bacillus 68 50 subtilis] 684 1
466 2 gnl.vertline.PID.vertline.e248400 orfRM1 gene product [Bacillus
subtilis] 68 40 693 1 2 787 gi.vertline.405804 transposase [Streptococcus
thermophilus] 68 46 700 2 772 596 gi.vertline.153801 enzyme scr-II
[Streptococcus mutans] 68 50 735 1 118 609 gi.vertline.969027
gamma-aminobutyrate permease [Bacillus 68 40 subtilis]
sp.vertline.P46349.vertline.GABP_BACSU GABA PERMEASE (4-AMINO BUTYRATE
TRANSPORT ARRIER) (GAMA-AMINOBUTYRATE PERMEASE). 750 1 2 529
gi.vertline.893358 PgsA [Bacillus subtilis] 68 54 762 2 1588 950
gi.vertline.1146240 ketopantoate hydroxymethyltransferase 68 49 [Bacillus
subtilis] 790 1 407 3 gi.vertline.142224 attachment protein ChvA (ttg strart
codon) 68 55 [Agrobacterium umefaciens] 882 1 3 278 gi.vertline.57572
glyceraldehyde-3-phosphate dehydrogenase 68 48 (NADP+) (phosphorylating)
attus rattus] 950 1 140 568 gi.vertline.882736 ORF_f278 [Escherichia coli] 68
53 969 2 554 339 gi.vertline.1118031 similar to neural cell adhesion
molecules 68 47 and neuroglians in their IG-like C2-type domains
[Caenorhabditis elegans] 970 1 297 73 gi.vertline.474404 cyclophilin
[Tolypocladium inflatum] 68 40 1 1 1103 3 gi.vertline.48790 ORF 3
[Pseudomonas putAda] 67 50 29 10 7156 6614
sp.vertline.P36672.vertline.PTTB_ECO PTS SYSTEM, **TREHALOSE**-SPECIFIC IIBC 67 52
LI COMPONENT (EIIBC-TRE) (**TREHALOSE**- PERMEASE IIBC COMPONENT)
(PHOSPHOTRANSFERASE ENZYME II, BC COMPONENT) (EC 2.7.1.69) (EII-TRE). 48 8
8035 9141 gi.vertline.975627 N-acylamino acid racemase [Amycolatopsis 67 48
sp.] 55 12 6621 7439 gi.vertline.391610 farnesyl diphosphate synthase
[Bacillus 67 47 stearothermophilus] pir.vertline.JX0257.vertline.JX0257
geranyltranstransferase (BC 2.5.1.10) - Bacillus tearothermophilus 57 13
13972 16401 gnl.vertline.PID.vertline.e255- 138 phenylalanyl-tRNA synthetase
beta subunit 67 47 [Bacillus subtilis] 63 4 1917 2729 gi.vertline.1321629

MIP related protein of E. coli 67 47 [Escherichia coli] 68 12 8600 8923
gi.vertline.793910 surface antigen [Homo sapiens] 67 43 72 7 7138 6740
gnl.vertline.PID.vertline.e209005 homologous to ORF2 in nrdEF operons of 67 39
E.coli and S.typhimurium [Lactococcus lactis] 72 10 8309 9433
gi.vertline.1199515 ferrous iron transport protein B 67 41 [Escherichia coli]
85 5 5315 4296 gi.vertline.142611 branched chain alpha-keto acid 67 52
dehydrogenase E1-alpha [Bacillus ubtilis] 101 5 4149 3100 gi.vertline.1109686
ProX [Bacillus subtilis] 67 48 110 4 2335 1292 gi.vertline.1066343
mu-crystallin [Homo sapiens] 67 48 114 12 12936 13520 gi.vertline.146218
serine hydroxymethyltransferase 67 50 [Escherichia coli] 115 5 3137 2010
gi.vertline.1256150 YbaR [Bacillus subtilis] 67 47 115 6 3199 2792
gi.vertline.1652593 hypothetical protein [Synechocystis sp.] 67 45 123 25
22739 24208 gi.vertline.148711 6-aminohexanoate-cyclic-dimer hydrolase 67 50
[Flavobacterium sp.] gi.vertline.488343 6- aminohexanoate-cyclic-dimer
hydrolase [Flavobacterium p.] 124 6 5139 4267 gi.vertline.1016770
prolipoprotein diacyiglyceryl transferase 67 50 [Staphylococcus aureus] 125
2 1306 221 gi.vertline.853743 L-alanoyl-D-glutamate peptidase 67 50
[Bacteriophage A118] 128 36 29462 28737 gi.vertline.142940 ftsA [Bacillus
subtilis] 67 46 138 27 17602 18183 gi.vertline.1256639 putative [Bacillus
subtilis] 67 50 138 31 21578 20097 gi.vertline.143245 Na+/H+ antiporter
[Bacillus firmus] 67 42 138 33 25165 23249 gi.vertline.1498811 M. jannaschii
predicted coding region 67 45 MJ0050 [Methanococcus jannaschii] 138 36 28690
27362 gnl.vertline.PID.vertline.e269549 Unknown [Bacillus subtilis] 67 47 144
4 3271 3717 gi.vertline.1753229 PKCI [Borrelia burgdorferi] 67 52 145 3 1435
2511 gi.vertline.1573615 ATP-binding protein (abc) [Haemophilus 67 47
influenzae] 146 5 4657 2804 gi.vertline.1045034 beta-galactosidase
[Xanthomonas campestris 67 51 pv. manihotis] 149 3 1978 1367
gi.vertline.806536 membrane protein [Bacillus 67 51 acidopullulyticus] 156 1
3 365 gnl.vertline.PID.vertline.e265539 ClpB-homologue [Thermus aquaticus 67
42 thermophilus] 158 15 14863 13766 gi.vertline.1573487 rbs repressor (rbsR)
[Haemophilus 67 40 influenzae] 158 17 16483 15959 gi.vertline.677850
hypothetical protein [Staphylococcus 67 51 aureus] 159 7 6872 6006
gi.vertline.1303949 YqiX [Bacillus subtilis] 67 41 159 9 8103 7498
gi.vertline.1303950 YqiY [Bacillus subtilis] 67 41 165 11 9846 9004
gi.vertline.606079 ORF_o267 [Escherichia coli] 67 36 169 2 2151 3047
gi.vertline.42371 pyruvate formate-lyase activating enzyme 67 44 (AA 1-246)
[Escherichia li] 179 13 13648 14451 gnl.vertline.PID.vertline.e257631
methyltransferase [Lactococcus lactis] 67 45 180 28 28656 29801
gi.vertline.666005 hypothetical protein [Bacillus subtilis] 67 48 194 6 2774
4231 gi.vertline.143245 Na+/H+ antiporter [Bacillus firmus] 67 41 194 10 6472
8259 gi.vertline.622991 mannitol transport protein [Bacillus 67 50
stearothermophilus] sp.vertline.P50852.vertline.PTMB_BACST PTS SYSTEM,
MANNITOL-SPECIFIC IIBC COMPONENT EIIBC-MTL) (MANNITOL- PERMEASE IIBC
COMPONENT) (PHOSPHOTRANSFERASE NZYME II, BC COMPONENT) (EC 2.7.1.69)
(EII-MTL). 204 5 1924 3006 gi.vertline.1235684 mevalonate pyrophosphate
decarboxylase 67 50 [Saccharomyces cerevisiae] 214 1 42 1196
gi.vertline.606013 CG Site No. 829 [Escherichia coli] 67 36 219 2 524 850
gnl.vertline.PID.vertline.e257628 ORF [Lactococcus lactis] 67 42 223 15 13640
14407 gi.vertline.496520 orf iota [Streptococcus pyogenes] 67 54 227 3 1011
1892 gi.vertline.1070013 protein-dependent [Bacillus subtilis] 67 37 233 12
9340 8339 gi.vertline.507880 xanthine dehydrogenase [Gallus gallus] 67 50 238
10 7951 9183 gi.vertline.1653948 hypothetical protein [Synechocystis sp.] 67
45 246 3 783 1430 gnl.vertline.PID.vertline.e233869 hypothetical protein

[Bacillus subtilis] 67 47 256 2 570 1601 gi.vertline.709992 hypothetical protein [Bacillus subtilisl 67 36 266 2 1266 835 gi.vertline.963038 ArpU [Enterococcus hirae] 67 42 285 1 3 809 gi.vertline.40014 pot. ORF 446 (aa 1-446) [Bacillus 67 53 subtilis] 288 10 6838 5801 gi.vertline.1651806 hypothetical protein [Synechocystis sp.] 67 45 301 10 8822 8562 gi.vertline.1303864 YqgQ [Bacillus subtilis] 67 43 312 5 2377 2595 gi.vertline.709991 hypothetical protein [Bacillus subtilis] 67 52 353 1 3 1472 gi.vertline.151259 HMG-CoA reductase (EC 1.1.1.88) 67 48 [Pseudomonas mevalonii] pir.vertline.A44756.vertline.A44756 hydroxymethylglutaryl-CoA reductase (EC 1.1.1.88) Pseudomonas sp. 359 2 984 439 gi.vertline.1773190 similar to E. coli yhaE [Escherichia coli] 67 45 359 3 2244 982 gi.vertline.1001478 hypothetical protein [Synechocystis sp.] 67 30 364 8 8469 7816 gi.vertline.496943 ORF [Saccharomyces cerevisiae] 67 50 386 12 6625 7833 gnl.vertline.PID.vertline.e254644 membrane protein [Streptococcus 67 36 pneumoniae] 394 2 497 2635 gnl.vertline.PID.vertline.e25593 hypothetical protein [Bacillus subtilis] 67 45 399 6 5410 3971 gi.vertline.665994 hypothetical protein

Detail Description Table CWU - DETL:

pir.vertline.S20433.vertline.S20433 lsp protein - Staphylococcus aureus sp.vertline.P31024.vertline.LSPA_STAAU LIPOPROTEIN SIGNAL PEPTIDASE (EC 3.4.23.36) PROLIPOPROTEIN SIGNAL PEPTIDASE) (SIGNAL PEPTIDASE II) (SPASE II). 221 7 2524 3468 gi.vertline.1353527 ORF10 [Bacteriophage rlt] 66 44 222 13 8272 8988 gi.vertline.466719 No definition line found [Eschenichia 66 48 coli] 223 18 15210 15971 gi.vertline.496520 orf iota [Streptococcus pyogenes] 66 57 232 5 3494 2715 gi.vertline.142706 comG1 gene product [Bacillus subtilis] 66 41 235 3 1774 734 gi.vertline.580897 OppB gene product [Bacillus subtilis] 66 47 244 2 906 1520 gi.vertline.15354 ORF 55.9 [Bacteriophage T4] 66 46 259 3 2355 1867 gi.vertline.56312 Gephyrin [Rattus norvegicus] 66 55 271 1 1 675 gi.vertline.1574748 tRNA pseudouridine 55 synthase (truB) 66 53 [Haemophilus influenzae] 277 1 1 927 gi.vertline.1303799 YqeN [Bacillus subtilis] 66 45 291 5 4587 3547 gnl.vertline.PID.vertline.e257609 sugar-binding transport protein 66 46 [Anaerocellum thermophilum] 292 25 20451 19912 gi.vertline.1649035 high-affinity periplasmic glutamine 66 50 binding protein [Salmonella typhimurium] 300 1 2302 77 gi.vertline.289262 comE ORF3 [Bacillus subtilis] 66 46 301 4 4290 3265 sp.vertline.P13226.vertline.GALE_S- TR UDP-GLUCOSE 4-EPIMERASE (EC 5.1.3.2) 66 51 LI (GALACTOWALDENASE). 301 5 4516 4689 gnl.vertline.PID.vertline.e212-164 PSII, protein N [Odontella sinensis] 66 58 314 1 360 4 gi.vertline.467452 unknown [Bacillus subtilis] 66 43 15 4 2559 2209 gi.vertline.1653498 ABC transporter [Synechocystis sp.] 66 44 320 3 2406 1081 gnl.vertline.PID.vertline.e250352 unknown [Mycobacterium tuberculosis] 66 35 332 2 157 921 gi.vertline.1303875 YghB [Bacillus subtilis] 66 44 334 2 1001 3076 gi.vertline.1651660 DNA ligase [Synechocystis sp.] 66 48 338 1 2 616 gi.vertline.845686 ORF-27 [Staphylococcus aureus] 66 54 338 7 5011 5496 gi.vertline.912476 No definition line found [Escherichia 66 48 coli] 341 5 1935 3107 gi.vertline.142538 aspartate aminotransferase [Bacillus sp.] 66 44 343 3 2548 2045 gnl.vertline.PID.vertline.e289147 similar to single strand binding protein 66 44 [Bacillus subtilis] 345 20 22093 22461 gi.vertline.1657795 dihydroneopterin aldolase 66 45 [Methylobacterium extorquens] 353 3 2621 2379 gnl.vertline.PID.vertline.e257628 ORF

[Lactococcus lactis] 66 52 365 4 5117 4779 gi.vertline.1742868 Mutator MutT protein (7,8-dihydro-8- 66 54 oxoguanine-triphosphatase) (8-oxo-dgtpase) (EC 3.6.1.-) (DGTP pyrophosphohydrolase). [Escherichia coli] 376 1 3 1076 gi.vertline.1778517 glycerol dehydrogenase homolog 66 45 [Escherichia coli] 394 7 5980 5648 gi.vertline.486358 ORF YKL202w [Saccharomyces cerevisiae] 66 38 421 4 1469 2539 gi.vertline.606375 ORF_f345 [Escherichia coli] 66 48 475 6 3978 3763 gi.vertline.532547 ORF14 [Enterococcus faecalis] 66 48 491 8 7710 7081 gi.vertline.1000453 TreR [Bacillus subtilis] 66 49 526 1 392 3 gi.vertline.1750125 xylulose kinase [Bacillus subtilis] 66 49 552 6 6147 5917 gi.vertline.1432152 PTS antiterminator [Klebsiella oxytoca] 66 37 571 2 560 1153 gi.vertline.1773132 multidrug resistance-like ATP-binding 66 38 protein Mdl [Esoherichia coli] 575 3 1075 539 gi.vertline.1651722 guanylate kinase [Synechocystis sp.] 66 48 608 2 631 113 gi.vertline.1213334 OrfX; hypothetical 22.5 KD protein 66 41 downstream of type IV prepilin leader peptidase gene; Method: conceptual translation supplied by author [Vibrio vulnificus] 640 1 877 2 sp.vertline.P50487.vertline.YCPX_CLO HYPOTHETICAL PROTEIN IN CPE 5'REGION 66 36 PE (FRAGMENT) 734 1 2 343 gi.vertline.1653602 hypothetical protein [Synechocystis sp.] 66 43 802 1 2 292 gnl.vertline.PID.vertline.e280516 voltage-gated sodium channel [Mus 66 58 musculus] 812 2 343 531 gi.vertline.511075 ORF2 [Streptococcus agalactiae] 66 51 823 1 1 393 gi.vertline.1303843 YqfV [Bacillus subtilis] 66 42 891 1 82 402 gi.vertline.567769 ORF5; predicted protein shows similarity 66 52 to ATP-binding transport roteins AmiE and AmiF of Streptococcus pneumoniae; disruptulon of RF5 leads to aminopterin resistance [Streptococcus parasanguis] 66 52 5 6 2630 3154 gi.vertline.1303811 YqeU [Bacillus subtilis] 65 50 16 1 2 628 gi.vertline.1742303 Acyl carrier protein phosphodiesterase 65 43 (ACP phosphodiesterase) (fragment), [Escherichia coli] 18 6 3360 2518 gi.vertline.601880 rep protein [Bacillus borstelensis] 65 40 21 11 7933 7706 gi.vertline.1500521 M. jannaschii predicted coding region 65 32 MJ1623 [Methanococcus jannaschii] 23 20 13459 13881 gi.vertline.488430 alcohol dehydrogenase 2 [Entamoeba 65 43 histolytica] 23 25 15987 16178 gnl.vertline.PID.vertline.e248966 F32D8.5 [Caenorhabditis elegans] 65 50 27 2 526 302 gi.vertline.1001644 regulatory components of sensory 65 44 transduction system [Synechocystis sp.] 29 9 6770 5727 sp.vertline.P36672.vertline.PTTB_ECO PTS SYSTEM, **TREHALOSE**-SPECIFIC IIBC 65 45 LI COMPONENT (EIIBC-TRE) (**TREHALOSE**- PERMEASE IIBC COMPONENT) (PHOSPHOTRANSFERASE ENZYME II, BC COMPONENT) (BC 2.7.1.69) (EII-TRE). 31 5 4611 5207 gi.vertline.171625 guanylate kinase [Saccharomyces 65 39 cerevisiae] 32 7 4085 3915 gi.vertline.150158 29 kD protein [Mycoplasma genitalium] 65 51 33 8 7396 7638 gi.vertline.1573421 protein translocation protein, low 65 26 temperature (secG) [Haemophilus influenzae] 35 1 2 499 gi.vertline.1737500 transcription antiterminator [Bacillus 65 40 stearothermophilus] 45 6 2537 3037 gi.vertline.511455 unknown [Coxiella burnetii] 65 37 46 3 1028 2254 gi.vertline.1001642 dGTP triphosphohydrolase [Synechocystis 65 43 sp.] 47 12 14524 14264 gi.vertline.150209 ORF 1 [Mycoplasma mycoides] 65 34 50 3 2866 2051 gi.vertline.1303830 YgfL [Bacillus subtilis] 65 40 57 11 12955 13332 gnl.vertline.PID.vertline.e254999 phenylalany-tRNA synthetase beta subunit 65 51 [Bacillus subtilis] 62 1 2 484 gi.vertline.1573470 H. influenzae predicted coding region 65 57 H10491 [Haemophilus influenzae] 68 1 49 282 gi.vertline.1573250 aspartate aminotransferase (aspC) 65 52 [Haemophilus influenzae] 72 2 567 1325 gi.vertline.466645 alternate name yhiD [Escherichia coli] 65 40 81 5 3711 2938 gi.vertline.1732200 PTS permease for mannose subunit IIPMan 65 43 [Vibria furnissii] 83 18 12506 12745

pir.vertline.D64042.vertl- ine.D64042 ribosomal-protein-alanine 65 50
acetyltransferase (riml) homolog - Haemophilus influenzae (strain Rd KW20)
100 38 28229 28032 gi.vertline.183075 glial fibrillary acidic protein [Homo 65
43 sapiens] 105 1 912 106 pir.vertline.S15248.vertline.YQBZCD fimC protein -
Dichelobacter nodosus 65 46 (serotype D) 106 5 6097 5102 gi.vertline.1143204
ORF2; Method: conceptual translation 65 44 supplied by author [Shigella
sonnei] 109 3 1165 899 gi.vertline.1573390 hypothetical [Haemophilus
influenzae] 110 7 5579 4257 pir.vertline.B44514.vertl- ine.B44514
hypothetical protein 1 (vnfA 5' region) - 65 43 Azotobacter vinelandii] 120 3
1249 1632 sp.vertline.P54746.vertlin- e.YBGB_ECO HYPOTHETICAL PROTEIN IN HRSA
3'REGION 65 48 LI (FRAGMENT). 122 2 896 1654 gi.vertline.1335913 unknown
[Erysipelothrix rhusiopathiae] 65 48 145 4 2509 3210 gi.vertline.1208965
hypothetical 23.3 kd protein [Eseherichia 65 40 coli] 149 7 4407 3502
gi.vertline.145173 35 kDa protein [Escherichia coli] 65 46 154 8 5738 4926
gi.vertline.405804 transposase [Streptococcus thermophilis] 65 47 155 1 306
512 gi.vertline.285627 E.coli SecE homologous protein [Bacillus 65 48
subtilis] pir.vertline.S39858.vertline.S39858 secE protein homolog - Bacillus
subtilis sp.vertline.Q06799.vertline.SECE- _BACSU PREPROTEIN TRANSLOCASE
SECE SUBUNIT. 158 1 150 1103 gi.vertline.289272 ferrichrome-binding protein
[Bacillus 65 40 subtilis] 158 16 14885 15946 gi.vertline.467172 add;
L308_C2_206 [Mycobacterium leprae] 65 36 173 4 2103 2912
gnl.vertline.PID.vertline.e254877 unknown [Mycobacterium tuberculosis] 65 41
173 12 9749 9054 gi.vertline.1652864 hypothetical protein [Synechocystis sp.]
65 50 179 16 15674 17035 gi.vertline.1171125 thioredoxin reductase
[Clostridium 65 41 litorale] 180 26 26911 28266
sp.vertline.P13692.vertline.P54_ENTF P54 PROTEIN PRECURSOR. 65 39 C 193 6
2893 3795 gi.vertline.39787 adaA [Bacillus subtilis] 65 45 194 5 1843 2238
gi.vertline.47394 5-oxoprolyl-peptidase [Streptococcus 65 48 pyogenes] 199 1
894 82 gi.vertline.1591118 nitrate transport ATP-binding protein 65 46
[Methanococcus jannaschii] 200 24 13441 13136 gi.vertline.144926 toxin A
[Clostridium difficile] 65 39 202 3 2925 1846 gi.vertline.413968 ipa-44d gene
product [Bacillus subtilis] 65 46 203 1 797 3 gi.vertline.1377832 unknown
[Bacillus subtilis] 65

Detail Description Table CWU - DETL:

[Azotobacter vinelandii] 60 31 34 4 6662 3279 gi.vertline.153952 polymerase
III polymerase subunit (dnaE) 60 37 [Salmonella typhimurium]
pir.vertline.A45915.vertline.A45915 DNA-directed DNA polymerase (EC 2.7.7.7)
III Ipha chain - Salmonella typhimurium 39 1 47 466 gi.vertline.1561567
Unknown [Bacillus subtilis] 60 35 39 4 1855 1361 gi.vertline.298045 Orf154
[Streptomyces ambofaciens] 60 41 48 4 2554 4128 gi.vertline.1255259
o-succinylbenzoic acid (OSB) CoA ligase 60 40 [Staphylococcus aureus] 56 9
6682 5795 gi.vertline.413940 ipa-16d gene product [Bacillus subtilis] 60 40
65 3 2105 2593 gi.vertline.1573061 hypothetical [Haemophilus influenzae] 60 34
72 9 7854 8330 gi.vertline.606343 CG Site No. 28964 [Escherichia coli] 60 39
81 3 2053 1406 gi.vertline.1574770 phenylalanyl-tRNA synthetase beta-subunit
60 46 (pheT) [Haemophilus influenzae] 81 4 2987 2130 gi.vertline.147404
mannose permease subunit II-M-Man 60 34 [Escherichia coli] 81 12 8280 7150
gnl.vertline.PID.vertline.e254984 hypothetical protein [Bacillus subtilis] 60
44 83 22 16887 16537 gi.vertline.509672 repressor protein [Bacteriophage
Tuc2009] 60 33 89 1 698 60 gi.vertline.840838 hypothetical 21.7 kDa protein

in ftsY 5' 60 36 region [Pseudomonas eruginosa] 89 12 12641 11856
gi.vertline.1377843 unknown [Bacillus subtilis] 60 40 89 17 18879 15844
gi.vertline.666069 orf2 gene product [Lactobacillus 60 37 leichmannii] 94 6
2281 3384 gi.vertline.468760 ORF334 [Rhizobium meliloti] 60 36 98 1 12 1970
gi.vertline.1652892 ABC transporter [Synechocystis sp.] 60 38 99 3 978 1460
gi.vertline.473955 DNA-binding protein [Lactobacillus sp.] 60 31 100 35 26818
26333 gi.vertline.347851 junctional sarcoplasmic reticulum 60 48 glycoprotein
[Oryctolagus uniculus] 100 45 30072 30449 gi.vertline.143547 Sin regulatory
protein (ttg start codon) 60 43 [Bacillus subtilis] gi.vertline.1303886 SinR
[Baciilus subtilis] 102 8 5923 6561 gi.vertline.1633572 Herpesvirus saimiri
ORF73 homolog 60 25 [Kaposi's sarcoma-associated herpes-like virus] 109 1
362 3 pir.vertline.S10655.vertline.S10655 hypothetical protein X - Pyrococcus
woesei 60 33 (fragment) 110 16 14806 14087
pir.vertline.JH0364.vertline.JH0364 hypothetical protein 176 (SAGP 5' region)
60 35 - Streptococcus pyogenes 110 20 18929 18414 gi.vertline.142450 ahrC
protein [Bacillus subtilis] 60 39 110 21 19124 19624 gi.vertline.142450 ahrC
protein [Bacillus subtilis] 60 40 111 1 289 2 gi.vertline.1256618 transport
protein [Bacillus subtilis] 60 31 122 7 5627 9589 gi.vertline.217191
5'-nucleotidase precursor [Vibrio 60 39 parahaemolyticus] 123 5 4390 3659
gi.vertline.1197667 vitellogenin [Anolis pulchellus] 60 27 123 20 18102 18407
gi.vertline.1303705 YrkF [Bacillus subtilis] 60 34 128 32 26229 25492
gi.vertline.1652485 hypothetical protein [Synechocystis sp.] 60 29 129 5 4421
6259 gi.vertline.1303853 YggF [Bacillus subtilis] 60 36 131 2 1112 2338
gi.vertline.699112 ugpC gene product [Mycobacterium leprae] 60 41 131 4 3194
4036 gi.vertline.296356 putative membrane transport protein 60 32
[Clostridium perfringens] pir.vertline.A56641.vertline.A56641 probable
membrane transport protein - Clostridium erfringens 131 8 6669 7901
gi.vertline.537054 2',3'-cyclic-nucleotide 2'- 60 40 phosphodiesterase
[Escherichia coli] pir.vertline.S56438.vertline.s56438 2',3'-cyclic-nucleotide
2-phosphodiesterase (EC .1.4.16) - Escherichia coli 133 11 9854 10240
gnl.vertline.PID.vertline.e249654 YneR [Bacillus subtilis] 60 37 138 7 6793
6263 gi.vertline.1486247 unknown [Bacillus subtilis] 60 48 146 4 2831 2328
gi.vertline.39979 P18 [Bacillus subtilis] 60 38 149 6 3504 3316
gi.vertline.145173 35 kDa protein [Escherichia coli] 60 47 154 5 2599 3558
gi.vertline.1773109 similar to S. typhimurium apbA 60 41 [Escherichia coli]
155 5 3061 4701 gi.vertline.388269 traC [Plasmid pAD1] 60 38 155 11 8565 8927
gi.vertline.1197460 MtfB [Escherichia coli] 60 39 158 10 11123 10032
gi.vertline.581809 tmbC gene product [Treponema pallidum] 60 39 165 7 6131
5700 gi.vertline.1439527 EIIA-man [Lactobacillus curvatus] 60 35 172 4 3169
3810 gi.vertline.1001342 hypothetical protein [Synechocystis sp.] 60 42 174 2
1574 762 gi.vertline.1045808 hypothetical protein (GB:U00021_19) 60 35
[Mycoplasma genitalium] 181 7 4975 4460 gi.vertline.683584 shikimate kinase
[Lactococcus lactis] 60 33 183 6 2719 2955 gi.vertline.1146198 ferredoxin
[Bacillus subtilis] 60 37 189 2 3528 2221 gi.vertline.396301 matches PS00041:
Bacterial regulatory 60 35 proteins, araC family ignature [Escherichia coli]
193 5 3121 2600 gi.vertline.39788 adaB [Bacillus subtilis] 60 49 195 11 4623
6569 gnl.vertline.PID.vertli- ne.e250887 potential coding region [Clostridium
60 39 difficile] 202 2 1837 1607 gi.vertline.693939 membrane ATPase
[Haloferax volcanii] 60 32 206 7 4794 3754 gi.vertline.1574702 hypothetical
[Haemophilus influenzae] 60 42 209 2 1308 433
pir.vertline.A38587.vertline.A38587 collagen, corneal - chicken (fragment) 60
51 220 3 4263 1213 gi.vertline.437706 alternative truncated translation
product 60 41 from E.coli [Streptococcus neumoniae] 222 9 6019 6522

gi.vertline.882463 protein-N(pi)-phosphohistidine-sugar 60 47 phosphotransferase [Escherichia oli] 222 12 8001 8336 gi.vertline.537035 ORF_o101 [Escherichia coli] 60 33 233 2 1294 827 gi.vertline.145091 flavodoxin [Desulfovibrio salexigens] 60 39 242 11 7370 7627 gi.vertline.1353404 cytochrome oxidase subunit I [Metridium 60 28 senile] 249 3 1109 1768 gi.vertline.143156 membrane bound protein [Bacillus subtilis] 60 41 251 3 4053 1933 gi.vertline.1235662 RfbC [Myxococcus xanthus] 60 42 256 4 2614 3867 gi.vertline.532612 ecotropic retrovirus receptor [Mus 60 37 musculus] 260 2 1539 802 gi.vertline.1208447 metahloprotease transporter [Serratia 60 35 marcescens] 261 5 4528 3179 gnl.vertline.PID.vertline.e246728 histidine kinase [Streptococcus gordonii] 60 25 269 3 2723 1563 gi.vertline.1591618 M. jannaschii predicted coding region 60 39 MJ0951 [Methanococcus jannaschii] 269 4 3541 2780 gi.vertline.1303794 YgeM [Bacillus subtilis] 60 36 269 11 7164 6595 gi.vertline.1303787 YgeG [Bacillus subtilis] 60 38 271 2 677 1651 gnl.vertline.PID.vertline- .e269877 riboflavin kinase [Bacillus subtilis] 60 43 271 3 1639 2247 gi.vertline.537148 ORF_f181 [Escherichia coli] 60 41 271 18 13502 13762 pir.vertline.S3934.vertline.S39341 grpE protein - Lactococcus lactis 60 40 277 2 1662 979 gi.vertline.1773109 similar to S. typhimurium apbA 60 41 [Escherichia coli] 279 13 10627 9773 gi.vertline.290545 f270 [Escherichia coli] 60 41 290 2 790 1695 gi.vertline.152886 elongation factor Ts (tsf) [Spiroplasma 60 38 citri] 291 4 3571 2612 gnl.vertline.PID.vertline.e257610 sugar-binding transport protein 60 40 [Anaerocellum thermophilum] 295 3 1309 2094 gi.vertline.1000453 TreR [Bacillus subtilis] 60 37 301 15 11063 11344 gi.vertline.535274 ORF1 [Streptococcus thermophilus] 60 36 310 3 2903 1266 gi.vertline.809765 aspartate aminotransferase (AA 1-402) 60 44 [Sulfolobus solfataricus] pir.vertline.S07088.vertline.S0708- 8 aspartate transaminase (EC 2.6.1.1) **- Sulfolobus** olfataricus 316 2 319 119 bbs.vertline.115298 polyprotein(coat protein) [raspberry 60 28 ringspot virus RRV, Peptide, 1107 aa] [Raspberry ringspot virus] 320 4 3085 2483 gi.vertline.143002 proton glutamate symnport protein [Bacillus 60 26 caldotenax] pir.vertline.S26246.vertline.S26246 glutamate/aspartate transport protein - Bacillus aldotenax 323 1 1 681 gi.vertline.1477486 transposase [Burkholderia cepacia] 60 44 330 4 3361 4488 gi.vertline.1778517 glycerol dehydrogenase homolog 60 48 [Escherichia coli] 356 3 2471 2205 gi.vertline.57633 neuronal myosin heavy chain [Rattus 60 40 rattus] 362 5 2458 2925 gnl.vertline.PID.vertline.e255090 hypothetical protein [Bacillus subtilis] 60 36 364 4 4096 5349 gi.vertline.1657522 hypothetical protein [Escherichia coli] 60 41 383 1 654 4 gn.vertline.PID.vertline.e288399 F56H6.k [Caenorhabditis elegans] 60 39 383 2 2208 853 gi.vertline.143536 sigma factor 54 [Bacillus subtilis] 60 37 386 2 130 510 gi.vertline.1046053 hypothetical protein (SP:P32049) 60 42 [Mycoplasma genitalium] 399 26 25892 27757 gi.vertline.895747 putative cel operon regulator [Bacillus 60 30 subtilis] 399 27 27721 28239 gi.vertline.146281 gut operon activator (gutM) [Escherichia 60 35 coli] 401 4 2081 3523 gi.vertline.142833 ORF2 [Bacillus subtilis] 60 36 405 2 1353 763 gi.vertline.633113 ORF3 [Streptococcus sobrinus] 60 42 407 7 4380 4589 gi.vertline.1674126 (AE000043) Mycoplasma pneumoniae, MG280 60 39 homolog, from M. genitalium [Mycoplasma pneumoniae] 408 1 12 539 gi.vertline.455006 orf6 [Rhodococcus fascians] 60 42 421 7 4113 3925 gi.vertline.60020 ORF31 (AA1-868) [Human herpesvirus 3] 60 43

PGPUB-DOCUMENT-NUMBER: 20020102696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102696 A1

TITLE: RECOMBINANT THERMOSTABLE ENZYME WHICH FORMS NON-REDUCING SACCHARIDE
FROM REDUCING AMYLACEOUS SACCHARIDE

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
MARUTA, KAZUHIKO	OKAYAMA		JP	
KUBOTA, MICHIO	OKYAMA		JP	
SUGIMOTO, TOSHIYUKI	OKAYAMA		JP	

APPL-NO: 09/ 419305

DATE FILED: October 15, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09419305 A1 19991015 parent continuation-of 08505448 19950721 US PATENTED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	190183/1994	1994JP-190183/1994	July 21, 1994
JP	189706/1995	1995JP-189706/1995	July 4, 1995

US-CL-CURRENT: 435/200,435/183

ABSTRACT:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pI of about 5.4-6.4, and forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

---------- KWIC ---------

Abstract Paragraph - ABTX:

Disclosed is a recombinant thermostable enzyme which has a molecular weight of about 69,000-79,000 daltons and a pI of about 5.4-6.4, and forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The enzyme has satisfactorily high thermostability, i.e. it is substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min, and this facilitates the production of such non-reducing saccharides on an industrial scale and in a satisfactorily-high yield.

Summary of Invention Paragraph - BSTX:

[0002] The present invention relates to a recombinant enzyme which forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3.

Summary of Invention Paragraph - BSTX:

[0004] **Trehalose** is a disaccharide which consists of 2 glucose molecules that are linked together with their reducing groups, and, naturally, it is present in fungi, algae, insects, etc., in an extremely small quantity. Having no reducing residue within the molecule, **trehalose** does not cause an unsatisfactory browning reaction even when heated in the presence of amino acids or the like, and because of this it can advantageously sweeten food products without fear of causing unsatisfactory coloration and deterioration. **Trehalose,** however, could not have been readily prepared in a desired amount by conventional production methods, so that it has not scarcely been used for sweetening food products.

Summary of Invention Paragraph - BSTX:

[0005] Conventional production methods are roughly classified into 2 groups, i.e. the one using cells of microorganisms and the other using a multi-enzymatic system where several enzymes are allowed to act on saccharides. The former, as disclosed in Japanese Patent Laid-Open No.154,485/75, is a method which comprises growing microorganisms such as bacteria and yeasts in nutrient culture media, and collecting **trehalose** mainly from the proliferated cells. The latter, as disclosed in Japanese Patent Laid-Open No.216,695/83, is a method which comprises providing maltose as a substrate, allowing a multi-enzymatic system using maltose- and **trehalose**-phosphorylases to act on maltose, and recovering the formed **trehalose** from the reaction system. The former facilitates the growth of microorganisms, but has a demerit that the content in the microorganisms is at most 15 w/w %, on a dry solid basis (d.s.b.). Although the latter can readily separate **trehalose,** it is

theoretically difficult to increase the **trehalose** yield by allowing such enzymes to act on substrates at a considerably-high concentration because the enzymatic reaction in itself is an equilibrium reaction of 2 different types of enzymes and the equilibrium point constantly inclines to the side of forming glucose phosphate.

Summary of Invention Paragraph - BSTX:

[0006] In view of the foregoing, the present inventors energetically screened enzymes which form non-reducing saccharides having a **trehalose** structure from amylaceous saccharides having a degree of glucose polymerization of at least 3, and have found that microorganisms such as those of the genera Rhizobium and Arthrobacter produce an absolutely novel enzyme which forms such non-reducing saccharides from such reducing amylaceous saccharides. They disclosed such an enzyme in Japanese Patent Application No.349,216/93. They also found that **trehalose** is readily formed from such non-reducing saccharides when glucoamylase or .alpha.-glucosidase acts on them.

Summary of Invention Paragraph - BSTX:

[0007] It was found that the enzymes produced from the aforesaid microorganisms have an optimum temperature of about 40.degree. C., and have some difficulties in their thermostability when used to prepare **trehalose**. It is recognized in this field that the recommendable temperature in the saccharification reaction of starch or amylaceous saccharides is one which exceeds 55.degree. C. because the contamination of microorganisms will occur at a temperature of 55.degree. C. or lower, decrease the pH of the reaction mixtures, and inactivate the enzymes used. Thus, a relatively-large amount of substrates remain intact. While the use of enzymes with a poor thermostability, a great care should be taken to control the pH, and, when the pH level lowers to extremely low level, alkalis should be added to reaction mixtures to increase the pH level as quickly as possible.

Summary of Invention Paragraph - BSTX:

[0008] In view of the foregoing, the present inventors screened thermostable enzyme with such a novel enzyme activity and have found that enzymes produced from microorganisms of the genus **Sulfolobus** including **Sulfolobus acidocaldarius** (ATCC 33909) are not substantially inactivated even when incubated at a temperature exceeding 55.degree. C., and they efficiently produce such non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides. These micro-organisms, however, are not sufficient in the enzyme productivity, and this requires a relatively-large scale culture to industrially produce non-reducing saccharides having a **trehalose** structure as an end unit.

Summary of Invention Paragraph - BSTX:

[0010] It is an object of the present invention to provide a recombinant

thermostable enzyme which forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides with a degree of glucose polymerization of at least 3 by using the recombinant DNA technology.

Summary of Invention Paragraph - BSTX:

[0015] It is another object of the present invention to provide a method for converting reducing amylaceous saccharides with a degree of glucose polymerization of at least 3 into non-reducing saccharides having a **trehalose** structure as an end unit.

Summary of Invention Paragraph - BSTX:

[0018] Forming non-reducing saccharides having a **trehalose** structure as an end unit from reducing saccharides having a degree of glucose polymerization of at least 3;

Summary of Invention Paragraph - BSTX:

[0029] The sixth object of the present invention is attained by a method for enzymatically converting reducing amylaceous saccharides which contains a step of allowing the recombinant thermostable enzyme to act on reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 to form non-reducing saccharides having a **trehalose** structure as an end unit.

Brief Description of Drawings Paragraph - DRTX:

[0030] FIG. 1 is a figure of the optimum temperature of a thermostable enzyme produced from **Sulfolobus acidocaldarius** (ATCC 33909).

Brief Description of Drawings Paragraph - DRTX:

[0031] FIG. 2 is a figure of the optimum pH of a thermostable enzyme produced from **Sulfolobus acidocaldarius** (ATCC 33909).

Brief Description of Drawings Paragraph - DRTX:

[0032] FIG. 3 is a figure of the thermostability of a thermostable enzyme produced from **Sulfolobus acidocaldarius** (ATCC 33909).

Brief Description of Drawings Paragraph - DRTX:

[0033] FIG. 4 is a figure of the pH stability of a thermostable enzyme produced from **Sulfolobus acidocaldarius** (ATCC 33909).

Detail Description Paragraph - DETX:

[0036] The recombinant thermostable enzyme according to the present invention forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 without inactivation even when allowed to react at a temperature exceeding 55.degree. C.

Detail Description Paragraph - DETX:

[0041] The present conversion method readily converts reducing amylaceous saccharide having a degree of glucose polymerization of at least 3 into non-reducing saccharides having a **trehalose** structure as an end unit.

Detail Description Paragraph - DETX:

[0042] The present invention has been accomplished based on the finding of a novel enzyme which forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Such an enzyme is obtainable from cultures of microorganisms of the species **Sulfolobus acidocaldarius** (ATCC 33909). The present inventors isolated such an enzyme by using in combination a various purification methods comprising column chromatography as a main technique, studied their properties and features, and revealed that the reality is a polypeptide with the following physicochemical properties:

Detail Description Paragraph - DETX:

[0044] Forming non-reducing saccharides having a **trehalose** structure as an end unit from reducing saccharides having a degree of glucose polymerization of at least 3;

Detail Description Paragraph - DETX:

[0057] The followings are experiments which were conducted to reveal the physicochemical properties of a thermostable enzyme produced from **Sulfolobus acidocaldarius** (ATCC 33909):

Detail Description Paragraph - DETX:

[0060] Into 500-ml flasks were put 100 ml aliquots of a liquid culture medium containing 0.1 w/v % polypeptone, 0.1 w/v % yeast extract, 0.2 w/v % ammonium sulfate, 0.05 w/v % potassium dihydrogen phosphate, 0.02 w/v % magnesium sulfate heptahydrate, 0.02 w/v % potassium chloride, and water, and the flasks were sterilized by autoclaving at 120.degree. C. for 20 min. After cooling the flasks a seed culture of **Sulfolobus acidocaldarius** (ATCC 33909) was inoculated into each liquid culture medium in each flask, followed by the incubation at 75.degree. C. for 24 hours under a rotary shaking condition of 130 rpm to

obtain a first seed culture. About 5 L of a fresh preparation of the same liquid culture medium was put in a 10-L fermenter, sterilized similarly as above, cooled to 75.degree. C., and adjusted to a pH 3.0, followed by inoculating one v/v % of the first seed culture into the sterilized liquid culture medium in the fermenter, and culturing the microorganisms at 75.degree. C. for 24 hours under an aeration condition of 500 ml/min. Thereafter, about 250 L of a fresh preparation of the same liquid culture medium was placed in a 300-L fermenter, sterilized similarly as above, cooled to 75.degree. C., and adjusted to a pH 3.0, followed by inoculating one v/v % of the second seed culture into the sterilized liquid culture medium, and culturing the microorganisms at 75.degree. C. for 42 hours under an aeration condition of 100 L/min.

Detail Description Paragraph - DETX:

[0071] The results in Table 1 show that the purified enzyme acted on reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 such as maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose to form non-reducing saccharides having a **trehalose** structure as an end unit such as .alpha.-glucosyltrehalose, .alpha.-maltosyltrehalose, .alpha.-maltotriosyltrehalose, .alpha.-maltotetraosyltrehalose and .alpha.-maltopentaosyltrehalose. In addition to these non-reducing saccharides and intact substrates, glucose and low molecular weight maltooligosaccharides as estimable hydrolysates of the substrates, were detected in the reaction mixtures, and this indicates that the purified enzyme has a hydrolytic activity. The yields of the non-reducing saccharides and hydrolysates from the substrates were respectively 30.2% and 27.6% for maltotriose, 65.4% and 18.4% for maltotetraose, and about 74-75% and about 2-3% for maltopentaose, maltohexaose and maltoheptaose. The purified enzyme formed non-reducing saccharides from maltooligosaccharides having a degree of glucose polymerization of at least 5 in a satisfactory yield, and less hydrolyzed the substrates, but did not newly form any saccharide from glucose and maltose.

Detail Description Paragraph - DETX:

[0097] A chromosomal DNA of **Sulfolobus acidocaldarius** (ATCC 33909) was screened by using an oligonucleotide as a probe which had been chemically synthesized based on the partial amino acid sequences in SEQ ID NOs:3 and 4, and this yielded a DNA fragment having a base sequence from the 5'-terminus consisting of about 2,200 base pairs in SEQ ID NO:2. The base sequence of the thermostable enzyme was decoded and revealing that it consists of 720 amino acids and has a partial amino acid sequence from the N-terminal in SEQ ID NO:1.

Detail Description Paragraph - DETX:

[0108] To 500-ml flasks were placed about 100 ml aliquots of a liquid culture medium consisting of 0.1 w/v % polypeptone, 0.1 w/v % yeast extract, 0.2 w/v % ammonium sulfate, 0.05 w/v % potassium dihydrogen phosphate, 0.02 w/v % magnesium sulfate heptahydrate, 0.02 w/v % potassium chloride, and water, and the flasks were sterilized by autoclaving at 120.degree. C. for 20 min,

cooled, and adjusted to a pH 3.0 by the addition of sulfate. A seed culture of **Sulfolobus acidocaldarius** (ATCC 33909) was inoculated into each flask, incubated at 75.degree. C. for 24 hours under a rotary shaking condition of 130 rpm to obtain a seed culture. About 5 L of a fresh preparation of the same liquid nutrient culture medium was placed in a 10-L fermenter, sterilized similarly as above, cooled to 75.degree. C., adjusted to a pH 3.0, and inoculated with one v/v % of the seed culture, followed by the incubation at 75.degree. C. for 24 hours under an aeration condition of 500 ml/min.

Detail Description Paragraph - DETX:

[0120] As a control, a seed culture of Escherichia coli XLI-Blue strain or **Sulfolobus acidocaldarius** (ATCC 33909) was inoculated into a fresh preparation of the same liquid culture medium but free of ampicillin. In the case of culturing **Sulfolobus acidocaldarius** (ATCC 33909), it was cultured and treated similarly as above except that the initial pH of the nutrient culture medium and the culturing temperature were respectively set to 3.0 and 75.degree. C. Assaying the resultant enzymatic activity, one L culture of **Sulfolobus acidocaldarius** (ATCC 33909) yielded about 1.8 units of the thermostable enzyme, and the yield was significantly lower than that of transformant ST35. Escherichia coli XLI-Blue strain used as a host did not form the thermostable enzyme.

Detail Description Paragraph - DETX:

[0121] Thereafter, the recombinant thermostable enzyme produced by the transformant ST35 was purified similarly as in Experiments 1 and 2 and examined for properties and features and revealing that it has substantially the same physicochemical properties of the thermostable enzyme from **Sulfolobus acidocaldarius** (ATCC 33909) because (i) the recombinant thermostable enzyme has a molecular weight of about 69,000-79,000 daltons on SDS-PAGE and an isoelectric point of about 5.4-6.4 on isoelectrophoresis, and (ii) it is not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min. These results indicate that the present thermostable enzyme can be prepared by the recombinant DNA technology with a significantly improved yield.

Detail Description Paragraph - DETX:

[0126] Analyses of the DNA fragments separated on the radiogram revealed that the complementary chain DNA contains the base sequence consisting of 2,200 base pairs in SEQ ID NO:5. An amino acid sequence that could be estimated from the base sequence was in SEQ ID NO:5, and it was compared with the partial amino acid sequences in SEQ ID NOs:3 and 4, and revealing that the partial amino acid sequence in SEQ ID NO:3 corresponded to that positioning from 1 to 30 in SEQ ID NO:5, and that in SEQ ID NO:4 corresponded to that positioning from 468 to 478 in SEQ ID NO:5. These results indicate that the present recombinant thermostable enzyme has the amino acid sequence from the N-terminal in SEQ ID NO:1, and, in the case of the DNA derived from **Sulfolobus acidocaldarius** (ATCC 33909), the amino acid sequence is encoded by the base sequence from the

5'-terminus in SEQ ID NO:2.

Detail Description Paragraph - DETX:

[0127] As is explained in the above, the thermostable enzyme, which forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3, was found as a result of the present inventors' long-term research. The thermostable enzyme has distinct physicochemical properties from those of other conventional enzymes. The present invention is to produce the thermostable enzyme by using the recombinant DNA technology. The present recombinant thermostable enzyme, as well as its preparation and uses, will be explained in detail with reference to the later described Examples.

Detail Description Paragraph - DETX:

[0128] The recombinant thermostable enzyme as referred to in the present invention means thermostable enzymes in general which are preparable by the recombinant DNA technology and capable of forming non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. Generally, the recombinant thermostable enzyme according to the present invention has a revealed amino acid sequence, and, as an example, the amino acid sequence from the N-terminal as shown in SEQ ID NO:1, and homologous ones to it can be mentioned. Variants having amino acid sequences homologous to the one in SEQ ID NO:1 can be obtained by replacing one or more bases in SEQ ID NO:1 with other bases without substantially alternating the inherent physicochemical properties. Although even when used the same DNA and it also depends on hosts into which the DNA is introduced, the ingredients and components of nutrient culture media for culturing transformants, and their cultivation temperature and pH, there may be produced modified enzymes which have the inherent physicochemical properties but defect one or more amino acids in SEQ ID NO:1, or those which have one or more amino acids added newly to the N-terminal after the DNA expression as the result of the modification of intracellular enzymes of the hosts. Such variants can be used in the present invention as long as they have the desired physicochemical properties.

Detail Description Paragraph - DETX:

[0130] The DNA usable in the present invention includes those are derived from natural resources and those which are artificially synthesized as long as they have the aforesaid base sequences. The natural resources for the DNA according to the present invention are, for example, microorganisms of the genus **Sulfolobus such as Sulfolobus acidocaldarius** (ATCC 33909), and from which genes containing the present DNA can be obtained. The aforementioned microorganisms can be inoculated in nutrient culture media and cultured for about 1-3 days under aerobic conditions, and the resultant cells were collected from the cultures and subjected to ultrasonication or treated with a cell-wall lysis enzyme such as lysozyme or .beta.-glucanase to extract genes containing the present DNA. In this case, a proteolytic enzyme such as protease can be used

along with the cell-wall lysis enzyme, and, when treated the cells with an ultrasonic disintegrator, they may be treated in the presence of a surfactant such as sodium dodecyl sulfate (SDS) or with freezing and thawing method. The objective DNA is obtainable by treating the resultant with phenol extraction, alcohol sedimentation, centrifugation, protease treatment and/or ribonuclease treatment generally used in this field. To artificially synthesize the present DNA, it can be chemically synthesized by using the base sequence in SEQ ID NO:2, or can be obtained in a plasmid form by inserting a DNA, which encodes the amino acid sequence in SEQ ID NO:1, into an appropriate self-replicable vector to obtain a recombinant DNA, introducing the recombinant DNA into an appropriate host to obtain a transformant, culturing the transformant, separating the proliferated cells from the resultant culture, and collecting plasmids containing the objective DNA from the cells.

Detail Description Paragraph - DETX:

[0133] The recombinant DNA thus obtained can be introduced into appropriate host microorganisms including Escherichia coli and those of the genus Bacillus as well as actinomyces and yeasts. In the case of using Escherichia coli as a host, the DNA can be introduced thereinto by culturing the host in the presence of the recombinant DNA and calcium ion, while in the case of using a microorganism of the genus Bacillus as a host the competent cell method and the colony hybridization method can be used. Desired transformants can be cloned by the colony hybridization method or by culturing a variety of transformants in nutrient culture media containing reducing amylaceous saccharides having a degree of glucose polymerization of at least 3, and selecting the objective transformants which form non-reducing saccharides having a **trehalose** structure as an end unit from the reducing amylaceous saccharides.

Detail Description Paragraph - DETX:

[0134] The transformants thus obtained intra- and extra-cellularly produce the objective enzyme when cultured in nutrient culture media. Generally, liquid culture media in general supplemented with carbon sources, nitrogen sources and minerals, and, if necessary, further supplemented with small amounts of amino acids and vitamins can be used in the invention. Examples of the carbon sources are saccharides such as unprocessed starch, starch hydrolysate, glucose, fructose, sucrose and **trehalose**. Examples of the nitrogen sources are organic- and inorganic-substances containing nitrogen such as ammonia and salts thereof, urea, nitrate, peptone, yeast extract, defatted soy been, corn steep liquor, and beef extract. Cultures containing the objective enzyme can be prepared by inoculating the transformants into nutrient culture media, and incubating them at a temperature of 20-65.degree. C. and a pH of 2-9 for about 1-6 days under aerobic conditions by the aeration-agitation method. Such cultures can be used intact as a crude enzyme, and, usually, cells in the cultures may be disrupted prior to use with ultrasonic and/or cell-wall lysis enzymes, followed by separating the thermostable enzyme from intact cells and cell debris by filtration and/or centrifugation and purifying the enzyme. The methods to purify the enzyme include conventional ones in general. From cultures intact cells and cell debris are eliminated and subjected to one or more methods such as concentration, salting out, dialysis, separatory

sedimentation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, gel electrophoresis and isoelectric point electrophoresis.

Detail Description Paragraph - DETX:

[0135] As is described above, the recombinant thermostable enzyme according to the present invention has a specific feature of forming non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 even when allowed to act on at a temperature exceeding 55.degree. C. The formed non-reducing saccharides have a satisfactorily mild and high-quality sweetness as well as an adequate viscosity and moisture-retaining ability, and, as a great advantageous feature, they can sweeten food products without fear of causing unsatisfactory coloration and deterioration because they have no reducing residue within their molecules. With these features a variety of amylaceous saccharides, which have been put aside because of their reducibilities, can be converted into saccharides which have a satisfactory handleability, usefulness, and no substantial reducibility or extremely-reduced reducibility.

Detail Description Paragraph - DETX:

[0137] In the enzymatic conversion method according to the present invention, the present recombinant thermostable enzyme is generally allowed to coexist in an aqueous solution containing one or more of the above reducing amylaceous saccharides as a substrate, followed by the enzymatic reaction at a prescribed temperature and pH until a desired amount of the objective reducing amylaceous saccharides is formed. Although the enzymatic reaction proceeds even below a concentration of 0.1 w/w %, d.s.b., of a substrate, a concentration of 2 w/w % or higher, d.s.b., preferably, in the range of 5-50 w/w %, d.s.b., of a substrate can be satisfactorily used when used the present conversion method in an industrial-scale production. The temperature and pH used in the enzymatic reaction are set to within the range of which does not inactivate the recombinant thermostable enzyme and allows the enzyme to effectively act on substrates, i.e. a temperature of higher than 55.degree. C. but not higher than 85.degree. C., preferably, a temperature in the range of about 56-70.degree. C., and a pH of 4-7, preferably, a pH in the range of about 5-6. The amount and reaction time suitable for the present recombinant thermostable enzyme are chosen depending on the enzymatic reaction condition. Thus, the present recombinant thermostable enzyme converts reducing amylaceous saccharides having a degree of glucose polymerization of at least 3 into non-reducing saccharides having a **trehalose** structure as an end unit, e.g. the conversion rate reaches up to about 74% when acts on maltopentaose.

Detail Description Paragraph - DETX:

[0139] The non-reducing saccharides thus obtained have a wide applicability to a variety of products which are apt to be readily damaged by the reducibility of saccharide sweeteners: For example, they can be satisfactorily used in food

products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant. Since the non-reducing saccharides almost qualitatively form **trehalose** when received an enzymatic action of a **trehalose**-releasing enzyme as disclosed in Japanese Patent Application No.79,291/94, they can be used as an intermediate for producing **trehalose** which could not have been readily prepared.

Detail Description Paragraph - DETX:

[0152] The purified enzyme was assayed for properties and features by the method in Experiment 2 and revealing that it had a molecular weight of about 69,000-79,000 daltons on SDS-PAGE and a pI of about 5.4-6.4 on isoelectrophoresis, and was not substantially inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min. These physicochemical properties were substantially the same as those of the enzyme from a donor microorganism of **Sulfolobus acidocaldarius** (ATCC 33909).

Detail Description Paragraph - DETX:

[0158] The product had a low DE of 4.8 and contained 12.8 w/w % .alpha.-glucosyltrehalose, 11.5 w/w % .alpha.-maltosyltrehalose, 46.6 w/w % .alpha.-maltotriosyltrehalose, 2.3 w/w % .alpha.-maltotetraosyltrehalos- e and 3.4 w/w % .alpha.-maltopentaosyl-**trehalose,** d.s.b. Similarly as the product in Example B-1, the product has a mild and moderate sweetness and an adequate viscosity and moisture-retaining ability, and can be satisfactorily used in compositions in general such as food products, cosmetics and pharmaceuticals as a sweetener, taste-improving agent, quality-improving agent, stabilizer, filler, excipient and adjuvant.

Detail Description Paragraph - DETX:

[0166] Conversion into Powdery Product Containing Crystalline **Trehalose**

Detail Description Paragraph - DETX:

[0167] Forty parts by weight of "PINE-DEX #4", a reducing amylaceous saccharide produced by Matsutani Chemical Ind., Co., Ltd., Kyoto, Japan, was dissolved in 60 parts by weight of water, and the solution was heated to 65.degree. C., adjusted to pH 5.5, and admixed with one unit/g reducing amylaceous saccharide, d.s.b., of a recombinant thermostable enzyme obtained by the method in Example A-1, followed by the enzymatic reaction for 96 hours. The reaction mixture was heated at 97.degree. C. for 30 min to inactivate the remaining enzyme, diluted up to a concentration of about 20 w/w %, d.s.b., and admixed with 10 units/g reducing amylaceous saccharide, d.s.b., of "GLUCOZYME", a glucoamylase specimen commercialized by Nagase Biochemicals, Ltd., Kyoto, Japan, followed by the enzymatic reaction for 40 hours. Thereafter, the reaction mixture was heated to inactivate the remaining enzyme, cooled, filtered, and, in usual manner, decolored with an activated charcoal, desalted and purified with an ion exchanger, and concentrated into an about 60 w/w % solution. The concentrate

with a **trehalose** content of 30.1 w/w %, d.s.b., was subjected to column chromatographic fractionation similarly as in Example B-2 except that "CG6000" , a strong-acid cation exchange resin in Na+-form commercialized by Japan Organo Co., Ltd., Tokyo, Japan, was used to obtain a fraction containing about 97 w/w % **trehalose**, d.s.b.

Detail Description Paragraph - DETX:

[0168] The fraction was concentrated up to about 75 w/w %, d.s.b., transferred to a crystallizer, and gradually cooled while stirring to obtain a massecuite with a crystallization percentage of about 45 w/w %, d.s.b. The massecuite was sprayed downward from a nozzle equipped on the upper part of a spraying tower at a pressure of about 150 kg/cm.sup.2 while an about 85.degree. C. hot air was blowing downward from the upper part of the spraying tower, and the formed crystalline powder was collected on a wire-netting conveyer provided on the basement of the drying tower and gradually conveyed out of the spraying tower while an about 45.degree. C. hot air was blowing to the crystalline powder from under the conveyer. The crystalline powder thus obtained was transferred to an ageing tower and aged for 10 hours in a hot air stream to complete the crystallization and drying. Thus, a powdery hydrous crystalline **trehalose** was obtained in a yield of about 90 w/w % to the material, d.s.b.

Detail Description Paragraph - DETX:

[0174] As is described above, the present invention is based on the finding of a novel thermostable enzyme which forms non-reducing saccharides having a **trehalose** structure as an end unit from reducing amylaceous saccharides having a degree of glucose polymerization of at least 3. The present invention is to explore a way to produce such a thermostable enzyme in an industrial scale and in a relatively-high efficiency by the recombinant DNA technology. The present conversion method using the recombinant thermostable enzyme readily converts non-reducing amylaceous saccharides, having a degree of glucose polymerization of at least 3, into non-reducing saccharides having a **trehalose** structure as an end unit without fear of causing bacterial contamination. The non-reducing saccharides have a mild and high-quality sweetness, and, because they have no reducing residue within their molecules, they can be advantageously incorporated into compositions in general such as food products, cosmetics and pharmaceuticals without fear of causing unsatisfactory coloration and deterioration. The present recombinant thermostable enzyme is the one with a revealed amino acid sequence, so that it can be used freely in the preparations of non-reducing saccharides having a **trehalose** structure as an end unit which are premised to be used in food products and pharmaceuticals.

Claims Text - CLTX:

1. A purified recombinant thermostable enzyme having the following physicochemical properties: (1) Action Forming non-reducing saccharides having a **trehalose** structure as an end unit and having a degree of glucose polymerization of at least 3 from maltotetraose or reducing amylaceous saccharides having a degree of glucose polymerization of at least 3; (2)

Molecular weight About 69,000-79,000 daltons on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); (3) Isoelectric point (pI) About 5.4-6.4 on isoelectrophoresis; (4) Thermostability Substantially not inactivated even when incubated in an aqueous solution (pH 7.0) at 85.degree. C. for 60 min.; and (5) Amino acid sequence An amino acid sequence which is not identical to SEQ ID NO:1 but is a functional equivalent thereof, and which contains one or more amino acid residues selected partially from SEQ ID NO:3 and SEQ ID NO:4.

PGPUB-DOCUMENT-NUMBER: 20020090618

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020090618 A1

TITLE: Thermostable reverse transcriptases and uses thereof

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Smith, Michael D.	Rockville	MD	US	
Potter, Robert Jason	Frederick	MD	US	
Dhariwal, Gulshan	Potomac	MD	US	
Gerard, Gary F.	Frederick	MD	US	
Rosenthal, Kim	Laytonsville	MD	US	

APPL-NO: 09/ 845157

DATE FILED: May 1, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60207196 20000526 US

US-CL-CURRENT: 435/6,435/199 ,435/5

ABSTRACT:

The present invention is in the fields of molecular and cellular biology. The invention is generally related to reverse transcriptase enzymes and methods for the reverse transcription of nucleic acid molecules, especially messenger RNA molecules. Specifically, the invention relates to reverse transcriptase enzymes which have been mutated or modified to increase thermostability, decrease terminal deoxynucleotidyl transferase activity, and/or increase fidelity, and to methods of producing, amplifying or sequencing nucleic acid molecules (particularly cDNA molecules) using these reverse transcriptase enzymes or compositions. The invention also relates to nucleic acid molecules produced by these methods and to the use of such nucleic acid molecules to produce desired polypeptides. The invention also concerns kits comprising such enzymes or compositions.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Appl. No. 60/207,196, filed May 26, 2000, the entire disclosure of which is incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0186] A variety of DNA polymerases are useful in accordance with the present invention. Such polymerases include, but are not limited to, Thermus thermophilus (Tth) DNA polymerase, Thermus aquaticus (Taq) DNA polymerase, Thermotoga neapolitana (Tne) DNA polymerase, Thermotoga maritima (Tma) DNA polymerase, Thermococcus litoralis (Tli or VENT.TM.) DNA polymerase, Pyrococcus furiosis (Pfu) DNA polymerase, Pyrococcus species GB-D (DEEPVENT.TM.) DNA polymerase, Pyrococcus woosii (Pwo) DNA polymerase, Bacillus sterothennophilus (Bst) DNA polymerase, Bacillus caldophilus (Bca) DNA polymerase, **Sulfolobus acidocaldarius** (Sac) DNA polymerase, Thermoplasma acidophilum (Tac) DNA polymerase, Thermus flavus (Tfl/Tub) DNA polymerase, Thermus ruber (Tru) DNA polymerase, Thermus brockianus (DYNAZYME.TM.) DNA polymerase, Methanobacterium thermoautotrophicum (Mth) DNA polymerase, Mycobacterium spp. DNA polymerase (Mtb, Mlep), and mutants, variants and derivatives thereof.

Detail Description Paragraph - DETX:

[0191] In addition to the enzyme components, the present compositions preferably comprise one or more buffers and cofactors necessary for synthesis of a nucleic acid molecule such as a cDNA molecule. Particularly preferred buffers for use in forming the present compositions are the acetate, sulfate, hydrochloride, phosphate or free acid forms of Tris-(hydroxymethyl)aminomethane (TRIS.RTM.), although alternative buffers of the same approximate ionic strength and pKa as TRIS.RTM. may be used with equivalent results. In addition to the buffer salts, cofactor salts such as those of potassium (preferably potassium chloride or potassium acetate) and magnesium (preferably magnesium chloride or magnesium acetate) are included in the compositions. Addition of one or more carbohydrates and/or sugars to the compositions and/or synthesis reaction mixtures may also be advantageous, to support enhanced stability of the compositions and/or reaction mixtures upon storage. Preferred such carbohydrates or sugars for inclusion in the compositions and/or synthesis reaction mixtures of the invention include, but are not limited to, sucrose, **trehalose,** glycerol, and the like. Furthermore, such carbohydrates and/or sugars may be added to the storage buffers for the enzymes used in the production of the enzyme compositions and kits of the invention. Such carbohydrates and/or sugars are commercially available from a number of sources, including Sigma (St. Louis, Mo.).

Detail Description Paragraph - DETX:

[0197] In another aspect, the compositions and reverse transcriptases of the invention may be prepared and stored in dry form in the presence of one or more carbohydrates, sugars, or synthetic polymers. Preferred carbohydrates, sugars or polymers for the preparation of dried compositions or reverse transcriptases include, but are not limited to, sucrose, **trehalose,** and polyvinylpyrrolidone (PVP) or combinations thereof. See, e.g., U.S. Pat. Nos. 5,098,893, 4,891,319, and 5,556,771, the disclosures of which are entirely incorporated

herein by reference. Such dried compositions and enzymes may be stored at various temperatures for extended times without significant deterioration of enzymes or components of the compositions of the invention. Preferably, the dried reverse transcriptases or compositions are stored at 4.degree. C. or at -20.degree. C.

PGPUB-DOCUMENT-NUMBER: 20020081581

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081581 A1

TITLE: COMPOSITIONS AND METHODS FOR REVERSE TRANSCRIPTION OF NUCLEIC ACID
MOLECULES

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GERARD, GARY F.	FREDERICK	MD	US	
SMITH, MICHAEL D.	ROCKVILLE	MD	US	
CHATTERJEE, DEB K.	NORTH POTOMAC	MD	US	

APPL-NO: 09/ 245026

DATE FILED: February 5, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09245026 A1 19990205 parent division-of 09064057 19980422 US PENDING
non-provisional-of-provisional 60044589 19970422 US
non-provisional-of-provisional 60049874 19970617 US

US-CL-CURRENT: 435/6,435/91.2

ABSTRACT:

The present invention is generally related to compositions and methods for the reverse transcription of nucleic acid molecules, especially messenger RNA molecules. Specifically, the invention relates to compositions comprising mixtures of polypeptides having reverse transcriptase (RT) activity, and to methods of producing, amplifying or sequencing nucleic acid molecules (particularly cDNA molecules) using these compositions or polypeptides, particularly at temperatures above about 55.degree. C. The invention also relates to nucleic acid molecules produced by these methods, to vectors and host cells comprising these nucleic acid molecules, and to the use of such nucleic acid molecules to produce desired polypeptides. The invention also relates to methods for producing Rous Sarcoma Virus (RSV) and Avian Myeloblastosis Virus (AMV) RTs or other Avian Sarcoma-Leukosis Virus (ASLV) RTs (.alpha. and/or .beta. subunits thereof), to isolated nucleic acid molecules encoding such RSV RT, AMV RT or other ASLV RT subunits, to vectors and host cells comprising these isolated nucleic acid molecules and to RSV RT, AMV RT

and other ASLV RT subunits produced by these methods. The invention further relates to nucleic acid molecules encoding recombinant heterodimeric RT holoenzymes, particularly heterodimeric RSV RTs, AMV RTs or other ASLV RTs (which may be .alpha..beta. RTs, .beta..beta. RTs, or .alpha. RTs), vectors (particularly baculovirus vectors) and host cells (particularly insect and yeast cells) comprising these nucleic acid molecules, methods for producing these heterodimeric RTs and heterodimeric RTs produced by these methods. The invention also relates to kits comprising the compositions, polypeptides, or RSV RTs, AMV RTs or other ASLV RTs of the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application Nos. 60/044,589, filed Apr. 22, 1997, and 60/049,874, filed Jun. 17, 1997, the disclosures of which are entirely incorporated by reference herein.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0115] A variety of DNA polymerases are useful in accordance with the present invention. Such polymerases include, but are not limited to, Thermus thermophilus (Tth) DNA polymerase, Thermus aquaticus (Taq) DNA polymerase, Thermotoga neapolitana (Tne) DNA polymerase, Thermotoga maritima (Tma) DNA polymerase, Thermococcus litoralis (Tli or VENT.TM.) DNA polymerase, Pyrococcus furiosis (Pfu) DNA polymerase, DEEPVENT.TM. DNA polymerase, Pyrococcus woosii (Pwo) DNA polymerase, Bacillus sterothermophilus (Bst) DNA polymerase, Bacillus caldophilus (Bca) DNA polymerase, **Sulfolobus acidocaldarius** (Sac) DNA polymerase, Thermoplasma acidophilum (Tac) DNA polymerase, Thermus flavus (Tfl/Tub) DNA polymerase, Thermus ruber (Tru) DNA polymerase, Thermus brockianus (DYNAZYME.TM.) DNA polymerase, Methanobacterium thermoautotrophicum (Mth) DNA polymerase, Mycobacterium spp. DNA polymerase (Mtb, Mlep), and mutants, variants and derivatives thereof.

Detail Description Paragraph - DETX:

[0121] In addition to the enzyme components, the present compositions preferably comprise one or more buffers and cofactors necessary for synthesis of a nucleic acid molecule such as a cDNA molecule. Particularly preferred buffers for use in forming the present compositions are the acetate, sulfate, hydrochloride, phosphate or free acid forms of Tris-(hydroxymethyl)aminomethane (TRIS.RTM.), although alternative buffers of the same approximate ionic strength and pKa as TRIS.RTM. may be used with equivalent results. In addition to the buffer salts, cofactor salts such as those of potassium (preferably potassium chloride or potassium acetate) and magnesium (preferably magnesium chloride or magnesium acetate) are included in the compositions. Addition of one or more carbohydrates and/or sugars to the compositions and/or synthesis reaction mixtures may also be advantageous, to support enhanced stability of the compositions and/or reaction mixtures upon storage. Preferred

such carbohydrates or sugars for inclusion in the compositions and/or synthesis reaction mixtures of the invention include, but are not limited to, sucrose, **trehalose,** and the like. Furthermore, such carbohydrates and/or sugars may be added to the storage buffers for the enzymes used in the production of the enzyme compositions and kits of the invention. Such carbohydrates and/or sugars are commercially available from a number of sources, including Sigma (St. Louis, Mo.).

Detail Description Paragraph - DETX:

[0127] In another aspect, the compositions and reverse transcriptases of the invention may be prepared and stored in dry form in the presence of one or more carbohydrates, sugars, or synthetic polymers. Preferred carbohydrates, sugars or polymers for the preparation of dried compositions or reverse transcriptases include, but are not limited to, sucrose, **trehalose,** and polyvinylpyrrolidone (PVP) or combinations thereof. See, e.g., U.S. Pat. Nos. 5,098,893, 4,891,319, and 5,556,771, the disclosures of which are entirely incorporated herein by reference. Such dried compositions and enzymes may be stored at various temperatures for extended times without significant deterioration of enzymes or components of the compositions of the invention. Preferably, the dried reverse transcriptases or compositions are stored at 4.degree. C. or at -20.degree. C.

Detail Description Paragraph - DETX:

[0186] Buffers. The pH of all buffers was determined at 23.degree. C., and buffers were stored at 4.degree. C. until use. Crack Buffer contained 50 mM Tris-HCl (pH 7.9), 0.5 M KCl, 0.02% (v/v) Triton X-100 and 20% (v/v) glycerol. Just before use, the following protease inhibitors (Boehringer Mannheim; Indianapolis, Ind.) were added to Crack Buffer at the final concentrations indicated: leupeptin (2 .mu.g/ml), Pefabloc (48 .mu.g/ml), pepstatin A (2 .mu.g/ml), benzamidine (800 .mu.g/ml) and PMSF (50 .mu.g/ml). Buffer A contained 20 mM Tris-HCl (pH 7.9), 0.25 M KCl, 0.02% (v/v) Triton X-100, and 10% (v/v) glycerol. Buffer B was Buffer A with 1 M imidazole added. Buffer S contained 50 mM Tris-HCl (pH 8.2), 0.02% (v/v) Triton X-100, 10% (v/v) glycerol, 0.1 mM EDTA and 1 mM dithiothreitol (DTT). Buffer T was Buffer S with 1 M KCl added. Buffer H contained 20 mM potassium phosphate (pH 7.1), 0.02% (v/v) Triton X-100, 20% (v/v) glycerol, 0.1 mM EDTA and 1 mM DTT. Buffer J was Buffer H with 1 M KCl added. Storage Buffer contained 200 mM potassium phosphate (pH 7.1), 0.05 % (v/v) NP-40,50% glycerol (v/v), 0.1 mM EDTA, 1 mM DTT, and 10% (w/v) **trehalose**.

PGPUB-DOCUMENT-NUMBER: 20010024793

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010024793 A1

TITLE: Nucleic acid-free thermostable enzymes and methods of production thereof

PUBLICATION-DATE: September 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Goldstein, Adam S.	New Market	MD	US	
Hughes, A. John JR.	Germantown	MD	US	

APPL-NO: 09/ 866816

DATE FILED: May 30, 2001

RELATED-US-APPL-DATA:

child 09866816 A1 20010530 parent continuation-of 09229967 19990114 US GRANTED parent-patent 6245533 US child 09229967 19990114 US parent continuation-of 08778082 19970102 US GRANTED parent-patent 5861295 US

US-CL-CURRENT: 435/6,435/183 ,435/194 ,435/91.1 ,435/91.2

ABSTRACT:

The present invention provides thermostable enzymes, such as DNA polymerases and restriction endonucleases, that are substantially free from contamination with nucleic acids. The invention also provides methods for the production of these enzymes, and kits comprising these enzymes which may be used in amplifying or sequencing nucleic acid molecules, including through use of the polymerase chain reaction (PCR).

---------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0006] These disruption approaches have several advantages, including their ability to rapidly and completely (in the case of physical methods) disrupt the bacterial cell such that the release of intracellular proteins is maximized. In fact, these approaches have been used in the initial steps of processes for the purification of a variety of bacterial cytosolic enzymes, including natural and recombinant proteins from mesophilic organisms such as Escherichia coli, Bacillus subtilis and Staphylococcus aureus (Laurent, S. J., and Vannier, F.

S., Biochimie 59:747-750 (1977); Cull, M., and McHenry, C. S., Meth. Enzymol. 182:147-153 (1990); Hughes, A. J., Jr., et al., J. Cell. Biochem. Suppl. 0 16 (Part B):84 (1992); Ausubel, F. M., et al., in: Current Protocols in Molecular Biology, New York: John Wiley & Sons, pp. 4.4.1-4.4.7 (1993)), as well as phosphatases, restriction enzymes, DNA or RNA polymerases and other proteins from thermophilic bacteria and archaea such as Thermus aquaticus, Thermus thermophilus, Thermus flavis, Thermus caldophilus, Thermotoga maritima, and **Sulfolobus acidocaldarius** (Shinomiya, T., et al., J. Biochem. 92(6):1823-1832 (1982); Elie, C., et al., Biochim. Biophys. Acta 951(2-3):261-267 (1988); Palm, P., et al., Nucl. Acids Res. 21(21):4904-4908 (1993); Park, J. H., et al., Eur. J. Biochem. 214(I):135-140 (1993); Harrell, R. A., and Hand, R. P., PCR Meth. Appl. 3(6):372-375 (1994); Meyer, W., et al., Arch. Biochem. Biophys. 319(I):149-156 (1995)).

Detail Description Paragraph - DETX:

[0021] Thermostable enzymes (e.g., DNA polymerases or restriction enzymes) may be prepared according to the methods of the present invention from a variety of thermophilic bacteria that are available commercially (for example, from American Type Culture Collection, Rockville, Md.). Suitable for use as sources of thermostable enzymes are the thermophilic bacteria Thermus aquaticus, Thermus thermophilus, Thermococcus litoralis, Pyrococcus furiosus, Pyrococcus woosii and other species of the Pyrococcus genus, Bacillus stearothermophilus, **Sulfolobus acidocaldarius,** Thermoplasma acidophilum, Thermus flavus, Thermus ruber, Thermus brockianus, Thermotoga neapolitana, Thermotoga maritima and other species of the Thermotoga genus, and Methanobacterium thermoautotrophicum, and mutants of each of these species. It will be understood by one of ordinary skill in the art, however, that any thermophilic microorganism may be used as a source for preparation of thermostable enzymes according to the methods of the present invention. Bacterial cells may be grown according to standard microbiological techniques, using culture media and incubation conditions suitable for growing active cultures of the particular species that are well-known to one of ordinary skill in the art (see, e.g., Brock, T. D., and Freeze, H., J. Bacteriol. 98(I):289-297 (1969); Oshima, T., and Imahori, K, Int. J. Syst. Bacteriol. 24(I):102-112 (1974)).

Detail Description Paragraph - DETX:

[0037] Following their purification, the substantially DNA-free thermostable enzymes may be stored until use in a buffered solution at temperatures of about -80.degree. to 25.degree. C., most preferably at -80.degree. to 4.degree. C., or in lyophilized form. Alternatively, the enzymes may be stabilized by drying in the presence of a sugar such as **trehalose** (U.S. Pat. Nos. 5,098,893 and 4,824,938) or acacia gum, pectin, carboxymethylcellulose, carboxymethylhydroxyethylcellulose, guar, carboxy guar, carboxymethylhydroxypropyl guar, laminaran, chitin, alginates or carrageenan. In addition, the enzymes provided by the present invention may be directly formulated into compositions to be used in techniques requiring the use of thermostable enzymes, such as compositions for nucleic acid sequencing or amplification in the case of thermostable DNA polymerases such as Taq, Tne, or Tma DNA polymerases, or mutants, derivatives or fragments thereof. These

formulations may be concentrated solutions of the enzymes, or solutions of the enzymes at working concentrations which may comprise additional components and which may be prepared as described in co-pending U.S. patent application Ser. No. 08/689,815, by Ayoub Rashtchian and Joseph Solus, entitled "Stable Compositions for Nucleic Acid Sequencing and Amplification," filed Aug. 14, 1996, which is incorporated by reference herein in its entirety.

US-PAT-NO: 6465236

DOCUMENT-IDENTIFIER: US 6465236 B1

TITLE: Thermostable collagen-digesting enzyme, novel microorganism producing the enzyme and process for producing the enzyme

DATE-ISSUED: October 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nishino; Tokuzo	Sendai	N/A	N/A	JP
Nakayama; Toru	Sendai	N/A	N/A	JP
Tsuruoka; Naoki	Sendai	N/A	N/A	JP
Akai; Minoru	Sendai	N/A	N/A	JP

APPL-NO: 09/ 807745

DATE FILED: April 18, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	11-242816	August 30, 1999

PCT-DATA:
APPL-NO: PCT/JP99/06392
DATE-FILED: November 16, 1999
PUB-NO: WO01/16302
PUB-DATE: Mar 8, 2001
371-DATE: Apr 18, 2001
102(E)-DATE: Apr 18, 2001

US-CL-CURRENT: 435/221; 435/219 ; 435/220 ; 435/252.1 ; 435/252.5

ABSTRACT:

Bacillus sp. NTAP-1 having been deposited under accession number FERM BP-6926; and a collagen-decomposing enzyme produced by bacterium. The above enzyme (1) has a capability of hydrolyzing, at the highest efficiency, collagen and gelatin from among casein, gelatin, albumin and collagen; (2) shows the optimum pH of 3.5 to 4.5; (3) shows the optimum temperature of 65 to 70.degree. C.; (4) after heating at 60.degree. C. at pH 6.0 for 4 hours, sustains an activity amounting to 60% or more of the level before the heat treatment; (5) remains stable at pH 3 to 6; and a molecular weight of approximately 46,000 when measured by SDS-PAGE.

4 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Detailed Description Text - DETX:

Does not decompose glycelol, erythritol, adonitol, .beta.-methyl-D-xylose, galactose, mannose, rhamnose, dulcitol, .alpha.-methyl-D-mannose, .alpha.-methyl-D-glucose, N-acetyl-glucosamine, amidagline, arbutin, aesuculin, salicin, cellobiose, maltose, milk sugar, melibiose, cane sugar, **trehalose,** inulin, melezitose, raffinose, glycogen, xylitol, gentiobiose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconic acid.

Detailed Description Text - DETX:

Among known species of the genus Bacillus, B. **acidocaldarius,** B. lichenformis, or B. coagulans are known to be thermophilic and acidophilic. However, this strain should not be B. lichenformis and B. coagulance because B. lichenformis and B. coagulance are catalase-positive and can grow at 40.degree. C. but not at 65.degree. C. Also, it is different from the standard species of B. **acidocaldarius** because it produces acetoin. Therefore, it is not possible to confirm that whether it is a modified species of B. **acidocaldarius** or it belongs to a different species; the species of this bacteria can not be specified.

US-PAT-NO: 6448049

DOCUMENT-IDENTIFIER: US 6448049 B1

TITLE: Starch conversion process

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsutsumi; Noriko	Chiba-ken	N/A	N/A	JP
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK

APPL-NO: 09/ 544123

DATE FILED: April 6, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional of application Ser. No. 09/129,075 filed on Aug. 4, 1998, now U.S. Pat. No. 6,087,149 which is a continuation of PCT/DK98/00304 filed Jul. 2, 1998, and claims priority under 35 U.S.C. 119 of Danish application no. 0787/97 filed on Jul. 2, 1997, and U.S. application Ser. No. 60/055,567 filed Aug. 13, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0787/97	February 7, 1997

US-CL-CURRENT: 435/98; 435/210

ABSTRACT:

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus and to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or **Sulfolobus,** to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

4 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Abstract Text - ABTX:

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus and to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or **Sulfolobus,** to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX:

The invention relates to a starch conversion process of the type which includes a debranching step. The invention also relates to the use of a thermostable isoamylase for debranching starch. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus, to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or **Sulfolobus,** to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX:

Starches such as corn, potato, wheat, manioc and rice starch are used as the starting material in commercial large scale production of sugars, such as high fructose syrup, high maltose syrup, maltodextrins amylose, **trehalose,** G2-G8 oligosaccharides (including functional oligosaccharides) and other carbohydrate products such as fat replacers.

Drawing Description Text - DRTX:

FIG. 5 shows the pH curve of **Sulfolobus acidocaldarius** isoamylase.

Detailed Description Text - DETX:

Specific examples of thermostable debranching enzymes are the thermostable isoamylases derived from the thermophilic bacteria such as **Sulfolobus acidocaldarius** ATCC 33909 (Maruta, K. et al., Biochimica et Biophysica Acta 1291, p. 177-181 (1996)), **Sulfolobus** solfataricus ATCC 35092 (accession number: Y08256) and Rhodothermus marinus DSM 4252 as will be described further below.

Detailed Description Text - DETX:

Isoamylases which can be used according to the invention include the thermostable isoamylase derived from the thermophilic archaebacteria **Sulfolobus acidocaldarius, Sulfolobus** solfataricus and the thermophilic eubacterium Rhodothermus marinus (as will be described in details below).

Detailed Description Text - DETX:

In the case of the starch conversion process is a starch depolymerization process the thermostable isoamylase is used in combination with an .alpha.-amylase during the liquefaction step. The thermostable isoamylase may be derived from the thermophilic archaebacteria **Sulfolobus acidocaldarius or Sulfolobus** solfataricus or from Rhodothermus marinus.

Detailed Description Text - DETX:

The homology search showed that the most related known sequence(s) were isoamylases from **Sulfolobus acidocaldarius and Sulfolobus** solfataricus. The DNA sequence of the invention (SEQ ID NO: 3) encoding an isoamylase shows about 51-52% DNA homology to the known isoamylase sequences from **Sulfolobus acidocaldarius and Sulfolobus** solfataricus and the corresponding amino acid sequence of the isoamylase of the invention (SEQ ID NO: 4) shows about 54-55% homology to a deduced amino acid sequence based on the known DNA sequence above.

Detailed Description Text - DETX:

Cloning of a DNA Sequence from a Strain of **Sulfolobus**

Detailed Description Text - DETX:

The invention also relates to a cloned DNA sequence encoding an enzyme with isoamylase activity derived from a strain of **Sulfolobus**. The DNA sequence may be derived from a strain of **Sulfolobus acidocaldarius or Sulfolobus** solfataricus.

Detailed Description Text - DETX:

The sequences may be the DNA sequences encoding an isoamylase from **Sulfolobus acidocaldarius** disclosed in Biochim. Biophys. Acta, 1291, p.117-181 (1996), it may be the DNA sequence from **Sulfolobus** solfataricus available in GeneBank under the Accession no. Y08256.

Detailed Description Text - DETX:

Cloning of the **Sulf lobus acidocaldarius** DNA sequence disclosed in Biochim. Biophys. Acta, 1291, p.177-181 (1996) is described below.

Detailed Description Text - DETX:

Cloning and Expression of **Sulfolobus acidocaldarius** Isoamylase Gene

Detailed Description Text - DETX:

The isoamylase gene from **Sulfolobus acidocaldarius** was cloned by PCR. The primers were designed based on the sequence shown in the article of Table 1. primer SINH: 5'-gtatatcaaagcttatgaaagatcgaccattaaagcctg-3' (SEQ ID NO:7) primer SICX: 5'-ggttgtctagatcactggaactctatcctcctgta-3' (SEQ ID NO:8)

Detailed Description Text - DETX:

Cloned gene from **Sulfolobus acidocaldarius** was inserted at Hind III and Xbal site of pJSOHX and expression vector named pFSI82 was obtained. pFSI 82 was transformed to Saccharomyces cerevisiae YNG318. pFSI82 map is shown in FIG. 4.

Detailed Description Text - DETX:

Characterization of the Cloned **Sulfolobus acidocaldarius** Isoamylase

Detailed Description Text - DETX:

The pH optimum of the **Sulfolobus acidocaldarius** isoamylase was determined. The Reaction was carried out at 70.degree. C. The checked pH was between pH 3.5-7.5. The pH optimum was determined to pH 5.5. The pH curve is shown in FIG. 5.

Detailed Description Text - DETX:

Temperature optimum of the **Sulfolobus** isoamylase was determined. The reaction was carried out at pH5.5 at between 40.degree.-90.degree. C. The optimum temperature was determined to be around 70.degree. C. The temperature curve is shown in FIG. 6.

Detailed Description Paragraph Table - DETL:

identity Origins of isoamylase genes DNA a.a. Pseudomonas amyloderamosa*1 50.4 % 33.0% Pseudomonas sp. SMP1*2 50.4 % 33.0% Flavobacterium sp.*3 54.0 % 34.1% Flavobacterium odoratum*4 54.8 % 36.6% **Sulfolobus** acidocaldarius*5 51.8

% 54.2% **Sulfolobus** solfataricus*6 51.1 % 55.0% *1: Table 1, 1 *2: Table 1, 2 *3: Table 1, 3 *4: Table 1, 4 *5: Table 1, 5 *6: GenBank: Accession number; Y08256

Detailed Description Paragraph Table - DETL:

TABLE 1 1 Pseudomonas amyloderamosa Biochim. Biophys. Acta, 1087, p. 309-315 (1990) 2 Pseudomonas sp. European patent publication num- ber: EP 0 302 838 A2 3 Flavobacterium sp. International patent publication number: W0 96/03513 4 Flavobacterium odoratum Japanese patent publication num- ber: JP08023981-A 5 **Sulfolobus acidocaldarius** Biochim. Biophys. Acta, 1291, p. 177-181 (1996)

Detailed Description Paragraph Table - DETL:

SEQUENCE LISTING <100> GENERAL INFORMATION: <160> NUMBER OF SEQ ID NOS: 16 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 1 <211> LENGTH: 22 <212> TYPE: DNA <213> ORGANISM: Pseudomonas amyloderamosa <220> FEATURE: <221> NAME/KEY: misc_feature <222> LOCATION: (0)...(0) <223> OTHER INFORMATION: N is inosine <400> SEQUENCE: 1 gnttyaaycc naayaarbtn yt 22 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 2 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Pseudomonas amyloderamosa <220> FEATURE: <221> NAME/KEY: misc_feature <222> LOCATION: (12)...(12) <223> OTHER INFORMATION: N is inosine <400> SEQUENCE: 2 ttratraccc cnatrtactg 20 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 3 <211> LENGTH: 2181 <212> TYPE: DNA <213> ORGANISM: Rhodothermus marinus <400> SEQUENCE: 3 atgtcacata gcgcgcaacc ggttacgtcg gtacaggccg tctggcccgg ccggccttat 60 ccgctgggtg ccacctggga cgggctgggc gtcaactttg ccctctacag ccagcacgcc 120 gaggcggtcg aactggtgct gttcgaccac ccggacgatc ccgcgccttc gcgcacgatc 180 gaagtgaccg aacggacagg cccgatctgg catgtgtacc tgcccggcct gcgtcccggc 240 cagctctacg gctatcgcgt ctacggaccc taccggccgg aggaaggcca ccgcttcaat 300 ccgaacaagg tgctgctcga cccctacgcg aaggccatcg gccggcccct tcgctggcac 360 gacagcctct tcggttacaa aatcggcgat ccggccgggg atctgtcgtt ctccgaagaa 420 gacagcgctc cgtacgcgcc gctgggagcc gtcgtggagg gctgtttcga gtggggcgac 480 gaccgcccgc cgcgcattcc ctgggaagac acgatcatct acgaaacgca cgtcaagggc 540 atcacgaagc tgcatccgga agtgccggag ccgctgcggg ggacgtatct ggggctgacc 600 tgcgagccgg tgctggagca cctgaagcag ctgggcgtca ccacgatcca gctccttccg 660 gtgcacgcaa aagtgcacga tcggcacctg gtcgagcgcg gcctgcgcaa ctactggggc 720 tacaatccgc tctgctactt tgcgccggag cccgagtacg ccacgaacgg gccgatctcg 780 gccgtgcgcg agttcaagat gatggtgcgg gcgctgcatg ctgccggctt cgaggtgatc 840 gtcgacgtgg tctacaacca cacgggcgaa ggcggcgtgc tgggccccac gctgtcgttc 900 cggggcatcg acaaccgcgc ctactacaag gccgatccga acaacccgcg ctttctggtc 960 gattacacgg gcaccggcaa cacgctggac gtgggcaacc cctacgtcat ccagctcatc 1020 atggacagcc tgcgctactg ggtcactgaa atgcacgtcg acggctttcg gttcgacctg 1080 gccgccgcgc tggcccgcga gctgtacgac gtggacatgc tctcgacctt ttttcaggtc 1140 attcagcagg acccggtgct cagccaggtc aagctcatcg ccgaaccctg ggacgtcggg 1200 ccgggggggt atcaggtggg acattttccc tggcagtgga ccgagtggaa cggccgctat 1260 cgtgacgccg tgcgccgctt ctggcggggc gatcggggcc tcaacggtga gtttgccacg 1320 cgctttgccg gctccagcga tctgtacgaa cgtagcggtc gtcgtccgtt cgcttcgatc 1380 aacttcgtca cggcgcacga cggcttcacg ctggaagacc tggtcagcta cacgaaaaag 1440 cacaacgaag

cgaatctgga aggcaaccgg gacggcatgg acgaaaacta cagcacgaac 1500 tgcggggtgg
agggacccac gcaggatccg tccgtgctgg cctgccggga agcgctcaag 1560 cgcagcctga
tcagcacgct ctttctctcg cagggcgtgc ccatgctgct gggcggcgac 1620 gagctgtcgc
gcacgcagca cggcaacaac aacgcctatt gccaggacaa cgagatcagc 1680 tggtacaact
ggcagctcga cacgcgcaag cagcagtttc tggagttcgt gcgccagacg 1740 atctggtttc
gcaagcagca tcggagcttc cggcgccgcc attttctgac cggattgccc 1800 aacggcggaa
ggccccgacg cagtctggtg gcacctgagg gtcggcccat gcgccacgag 1860 gactggacca
acccggagct gacggccttc ggactgctgc tgcacggcga cgccattcag 1920 gggaccgacg
agcacggacg accgtttcgc gacgacacgt ttctgattct gttcaacaac 1980 ggcagcgaag
ccgtgccggt cgtggtgccg gaggtatgct cctgtggcaa gccgcaccac 2040 tgggaggtgg
tcccggtgtt tcaacgcaat gtggagcccc ccacgtgcgc gcccggcgag 2100 acgctgtcgc
tcccgcccgg cgtgctgacg gtgctggtgg ccgtaccgcc gttctcggat 2160 ggaaacacgg
agccggcctg a 2181 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO
4 <211> LENGTH: 726 <212> TYPE: PRT <213> ORGANISM:
Rhodothermus marinus <400> SEQUENCE: 4 Met Ser His Ser Ala Gln Pro Val
Thr Ser Val Gln Ala Val Trp Pro 1 5 10 15 Gly Arg Pro Tyr Pro Leu Gly Ala Thr
Trp Asp Gly Leu Gly Val Asn 20 25 30 Phe Ala Leu Tyr Ser Gln His Ala Glu Ala
Val Glu Leu Val Leu Phe 35 40 45 Asp His Pro Asp Asp Pro Ala Pro Ser Arg Thr
Ile Glu Val Thr Glu 50 55 60 Arg Thr Gly Pro Ile Trp His Val Tyr Leu Pro Gly
Leu Arg Pro Gly 65 70 75 80 Gln Leu Tyr Gly Tyr Arg Val Tyr Gly Pro Tyr Arg
Pro Glu Glu Gly 85 90 95 His Arg Phe Asn Pro Asn Lys Val Leu Leu Asp Pro Tyr
Ala Lys Ala 100 105 110 Ile Gly Arg Pro Leu Arg Trp His Asp Ser Leu Phe Gly
Tyr Lys Ile 115 120 125 Gly Asp Pro Ala Gly Asp Leu Ser Phe Ser Glu Glu Asp
Ser Ala Pro 130 135 140 Tyr Ala Pro Leu Gly Ala Val Val Glu Gly Cys Phe Glu
Trp Gly Asp 145 150 155 160 Asp Arg Pro Pro Arg Ile Pro Trp Glu Asp Thr Ile
Ile Tyr Glu Thr 165 170 175 His Val Lys Gly Ile Thr Lys Leu His Pro Glu Val
Pro Glu Pro Leu 180 185 190 Arg Gly Thr Tyr Leu Gly Leu Thr Cys Glu Pro Val
Leu Glu His Leu 195 200 205 Lys Gln Leu Gly Val Thr Thr Ile Gln Leu Leu Pro
Val His Ala Lys 210 215 220 Val His Asp Arg His Leu Val Glu Arg Gly Leu Arg
Asn Tyr Trp Gly 225 230 235 240 Tyr Asn Pro Leu Cys Tyr Phe Ala Pro Glu Pro
Glu Tyr Ala Thr Asn 245 250 255 Gly Pro Ile Ser Ala Val Arg Glu Phe Lys Met
Met Val Arg Ala Leu 260 265 270 His Ala Ala Gly Phe Glu Val Ile Val Asp Val
Val Tyr Asn His Thr 275 280 285 Gly Glu Gly Gly Val Leu Gly Pro Thr Leu Ser
Phe Arg Gly Ile Asp 290 295 300 Asn Arg Ala Tyr Tyr Lys Ala Asp Pro Asn Asn
Pro Arg Phe Leu Val 305 310 315 320 Asp Tyr Thr Gly Thr Gly Asn Thr Leu Asp
Val Gly Asn Pro Tyr Val 325 330 335 Ile Gln Leu Ile Met Asp Ser Leu Arg Tyr
Trp Val Thr Glu Met His 340 345 350 Val Asp Gly Phe Arg Phe Asp Leu Ala Ala
Ala Leu Ala Arg Glu Leu 355 360 365 Tyr Asp Val Asp Met Leu Ser Thr Phe Phe
Gln Val Ile Gln Gln Asp 370 375 380 Pro Val Leu Ser Gln Val Lys Leu Ile Ala
Glu Pro Trp Asp Val Gly 385 390 395 400 Pro Gly Gly Tyr Gln Val Gly His Phe
Pro Trp Gln Trp Thr Glu Trp 405 410 415 Asn Gly Arg Tyr Arg Asp Ala Val Arg
Arg Phe Trp Arg Gly Asp Arg 420 425 430 Gly Leu Asn Gly Glu Phe Ala Thr Arg
Phe Ala Gly Ser Ser Asp Leu 435 440 445 Tyr Glu Arg Ser Gly Arg Arg Pro Phe
Ala Ser Ile Asn Phe Val Thr 450 455 460 Ala His Asp Gly Phe Thr Leu Glu Asp
Leu Val Ser Tyr Thr Lys Lys 465 470 475 480 His Asn Glu Ala Asn Leu Glu Gly
Asn Arg Asp Gly Met Asp Glu Asn 485 490 495 Tyr Ser Thr Asn Cys Gly Val Glu
Gly Pro Thr Gln Asp Pro Ser Val 500 505 510 Leu Ala Cys Arg Glu Ala Leu Lys
Arg Ser Leu Ile Ser Thr Leu Phe 515 520 525 Leu Ser Gln Gly Val Pro Met Leu
Leu Gly Gly Asp Glu Leu Ser Arg 530 535 540 Thr Gln His Gly Asn Asn Asn Ala
Tyr Cys Gln Asp Asn Glu Ile Ser 545 550 555 560 Trp Tyr Asn Trp Gln Leu Asp
Thr Arg Lys Gln Gln Phe Leu Glu Phe 565 570 575 Val Arg Gln Thr Ile Trp Phe
Arg Lys Gln His Arg Ser Phe Arg Arg 580 585 590 Arg His Phe Leu Thr Gly Leu
Pro Asn Gly Gly Arg Pro Arg Arg Ser 595 600 605 Leu Val Ala Pro Glu Gly Arg

Pro Met Arg His Glu Asp Trp Thr Asn 610 615 620 Pro Glu Leu Thr Ala Phe Gly Leu Leu Leu His Gly Asp Ala Ile Gln 625 630 635 640 Gly Thr Asp Glu His Gly Arg Pro Phe Arg Asp Asp Thr Phe Leu Ile 645 650 655 Leu Phe Asn Asn Gly Ser Glu Ala Val Pro Val Val Val Pro Glu Val 660 665 670 Cys Ser Cys Gly Lys Pro His His Trp Glu Val Val Pro Val Phe Gln 675 680 685 Arg Asn Val Glu Pro Pro Thr Cys Ala Pro Gly Glu Thr Leu Ser Leu 690 695 700 Pro Pro Gly Val Leu Thr Val Leu Val Ala Val Pro Pro Phe Ser Asp 705 710 715 720 Gly Asn Thr Glu Pro Ala 725 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 5 <211> LENGTH: 26 <212> TYPE: DNA <213> ORGANISM: Rodethermus marinus <400> SEQUENCE: 5 gtcagtagcc catggcacat tagcgc 26 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 6 <211> LENGTH: 24 <212> TYPE: DNA <213> ORGANISM: Rodethermus marinus <400> SEQUENCE: 6 ctcggccgga ctagatctgt cttc 24 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 7 <211> LENGTH: 39 <212> TYPE: DNA <213> ORGANISM: **Sulfolobus acidocaldarius** <400> SEQUENCE: 7 gtatatcaaa gcttatgaaa gatcgaccat taaagcctg 39 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 8 <211> LENGTH: 35 <212> TYPE: DNA <213> ORGANISM: **Sulfolobus acidocaldarius** <400> SEQUENCE: 8 ggttgtctag atcactggaa ctctatcctc ctgta 35 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 9 <211> LENGTH: 26 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: mutation <222> LOCATION: (0)...(0) <223> OTHER INFORMATION: Oligo 1 <400> SEQUENCE: 9 gatcaagctt ggaattcgct cgagct 26 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 10 <211> LENGTH: 26 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <221> NAME/KEY: mutation <222> LOCATION: (0)...(0) <223> OTHER INFORMATION: Oligo 2 <400> SEQUENCE: 10 ctagagctcg agcgaattcc aagctt 26 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 11 <211> LENGTH: 140 <212> TYPE: PRT <213> ORGANISM: Flavobacterium sp. <400> SEQUENCE: 11 Phe Asn Pro Asn Lys Leu Leu Leu Asp Pro Tyr Ala Lys Ala Val His 1 5 10 15 Arg Gln Ile Asp Trp Asp Pro Ala Leu Phe Ser Tyr Asn Leu Gly Asp 20 25 30 Pro Asp Ser Arg Asn Asp Asp Asp Ser Ala Pro His Met Met Leu Gly 35 40 45 Val Val Ile Asn Pro Phe Phe Asp Trp Asp Gly Asp Lys Leu Pro Arg 50 55 60 Ile Pro Tyr His Lys Ser Val Ile Tyr Glu Ala His Val Lys Gly Leu 65 70 75 80 Thr Gln Leu His Pro Glu Val Pro Glu Gly Ala Ala Arg Tyr Tyr Ala 85 90 95 Gly Val Ala His Pro Ala Val Ile Ser His Leu Gln Lys Leu Gly Ile 100 105 110 Thr Ala Ile Glu Leu Met Pro Val His Gln Phe Val Asn Asp Gly Ile 115 120 125 Leu Gln Asp Lys Gly Leu Asn Asn Tyr Trp Gly Tyr 130 135 140 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 12 <211> LENGTH: 143 <212> TYPE: PRT <213> ORGANISM: Flavobacterium devorans <400> SEQUENCE: 12 Phe Asn Pro Asn Lys Val Leu Leu Asp Pro Tyr Ala Arg Lys Leu Phe 1 5 10 15 Gly Glu Ile Lys Trp Thr Asp Ala Leu His Gly Tyr Gln Ile Arg Ser 20 25 30

US-PAT-NO: 6410241

DOCUMENT-IDENTIFIER: US 6410241 B1

TITLE: Methods of screening open reading frames to determine whether they encode polypeptides with an ability to generate an immune response

DATE-ISSUED: June 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sykes; Kathryn F.	Dallas	TX	N/A	N/A
Johnston; Stephen Albert	Dallas	TX	N/A	N/A

APPL-NO: 09/ 535366

DATE FILED: March 24, 2000

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application Ser. No. 60/125,864, filed Mar. 24, 1999, and U.S. Provisional Application Ser. No. 60/127,222, filed Mar. 31, 1999, each of which disclosures is specifically incorporated herein by reference in its entirety.

US-CL-CURRENT: 435/6; 435/7.21

ABSTRACT:

The present invention relates to linear expression elements (LEEs) and circular expression elements (CEEs), which are useful in a variety of molecular biology protocols. Specifically, the invention relates to the use of LEEs and CEEs to screen for gene function, biological effects of gene function, antigens, and promoter function. The invention also provides methods of producing proteins, antibodies, antigens, and vaccines. Also, the invention relates to methods of making LEEs and CEEs, and LEEs and CEEs produced by such methods.

28 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX:

In certain embodiments, the organism is an archaea (a.k.a. archaebacteria; e.g., a methanogens, a halophiles, a **sulfolobus**). In particular embodiments, the archaea may be, but is not limited to, a korarchaeota; a crenarchaeota, including but not limited to, a thermofilum, a pyrobaculum, a thermoproteus, a **sulfolobus,** a metallosphaera, an acidianus, a thermodiscus, a igneococcus, a thermosphaera, a desulfurococcus, a staphylothermus, a pyrolobus, a hyperthermus or a pyrodictium; or an euryarchaeota, including but not limited to a halobacterilaes, methanomicrobilaes, a methanobacteriales, a methanococcales, a methanopyrales, an archeoglobales, a thermoplasmales or a thermococcales.

Detailed Description Text - DETX:

A preferred adjuvant in the present invention is BCG BCG (bacillus Calmette-Guerin, an attenuated strain of Mycobacterium) and BCG-cell wall skeleton (CWS) may also be used as adjuvants in the invention, with or without **trehalose** dimycolate. **Trehalose** dimycolate may be used itself. Azuma et al., (1988) show that **trehalose** dimycolate administration correlates with augmented resistance to influenza virus infection in mice. **Trehalose** dimycolate may be prepared as described in U.S. Pat. No. 4,579,945.

Detailed Description Text - DETX:

The detoxified endotoxins may be combined with other adjuvants to prepare multi-adjuvant-incorporated cells. Combination of detoxified endotoxins with **trehalose** dimycolate is contemplated, as described in U.S. Pat. No. 4,435,386. Combinations of detoxified endotoxins with **trehalose** dimycolate and endotoxic glycolipids is also contemplated (U.S. Pat. No. 4,505,899), as is combination of detoxified endotoxins with cell wall skeleton (CWS) or CWS and **trehalose** dimycolate, as described in U.S. Pat. Nos. 4,436,727, 4,436,728 and 4,505,900. Combinations of just CWS and **trehalose** dimycolate, without detoxified endotoxins, is also envisioned to be useful, as described in U.S. Pat. No. 4,520,019.

Detailed Description Text - DETX:

Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, .gamma.-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, **trehalose** dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

US-PAT-NO: 6403355

DOCUMENT-IDENTIFIER: US 6403355 B1

TITLE: Amylases

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hagihara; Hiroshi	Tochigi	N/A	N/A	JP
Kitayama; Kaori	Tochigi	N/A	N/A	JP
Hayashi; Yasuhiro	Tochigi	N/A	N/A	JP
Igarashi; Kazuaki	Tochigi	N/A	N/A	JP
Endo; Keiji	Tochigi	N/A	N/A	JP
Ozaki; Katsuya	Tochigi	N/A	N/A	JP

APPL-NO: 09/ 465519

DATE FILED: December 16, 1999

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	10-362487	December 21, 1998
JP	10-362488	December 21, 1998

US-CL-CURRENT: 435/202; 435/183 ; 435/200 ; 435/201 ; 435/69.1

ABSTRACT:

Described are liquefying alkaline amylases each having residual activity not less than 70% when treated at pH 10 and 45.degree. C. for 30 minutes in the presence of 1 to 100 mM of EDTA or EGTA; and a detergent comprising the same. Compared with the conventional amylases for a detergent, they have high chelating-agent resisting performance.

10 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Brief Summary Text - BSTX:

Among the liquefying amylases known to date, a liquefying .alpha.-amylase (WO90/11352) derived from the strain belonging to Pyrococcus sp. and an

.alpha.-amylase (WO96/02633) which is derived from the strain belonging to **Sulfolobus** sp. and is effective in the liquefying step of a starch are free from the influence from a chelating agent. These enzymes however have the optimum acting pH in a range of 4 to 6 and 2.5 to 4.5, respectively and do not act in the alkaline range so that they are not suited as a component of a detergent.

Detailed Description Paragraph Table - DETL:

TABLE I Strain KSM-K36 Stain KSM-K38 (a) Results of The strains K36 and K38 are bacilli having a size of 1.0 to 1.2 .mu.m .times. microscopic observation 2.4 to 5.4 .mu.m and 1.0 to 1.2 .mu.m .times. 1.8 to 3.8 .mu.m, respectively. They form an oval endospore (1.0 to 1.2 .mu.m .times. 1.2 to 1.4 micron) at the center or near the end of the cell. Positive in the Gram's stain. Having no acid resistance. (b) Growth in various media Since the present strain is alkaliphilic, 0.5% sodium carbonate is added to the medium employed in the following tests. Nutrient agar plate Good growth is observed. The Good growth is observed. The culture colony has a circular shape. It colony has a circular shape. It has a flat surface, but a rough has a flat surface and a smooth periphery. The color of the periphery. The color of the colony is pale earthlike color. colony is yellowish brown. Nutrient agar slant Growth is observed. Growth is observed. culture Nutrient broth cutture Growth is observed. Growth is observed. Nutrient-gelatin Good growth is observed. No Good growth is observed. No stab culture liquefaction of gelatin is liquefaction of gelatin is observed. observed. Litmus milk No change is observed. No change is observed. (c) Physiological properties Reduction of a Reduction of a nitrate is positive. Reduction of a nitrate is positive. nitrate and Denitrification reaction is Denitrification reaction is denitrification negative. negative. reaction MR test Owing to the alkaline medium, Owing to the alkaline medium, judgment is impossible. judgment is impossible. V-P test Negative. Negative. Formation of indole Negative. Negative. Formation of hydrogen Negative. Negative. nitride Hydrolysis of starch Positive. Positive. Assimilation of It grows on a Christensen's It grows on a Christensen's citric acid medium, but not on a Koser's medium, but not on a Koser's medium and Simmon's medium. medium and Simmon's medium. Assimilation of It assimilates a nitrate but not an It assimiiates a nitrate but not an an inorganic ammonium salt. ammonium salt. nitrogen source Formation of a colorant Formation of a pale yellow Negative. colorant on King's B medium. Urease Negative. Negative. Oxidase Negative. Negative. Catalase Positive. Positive. Range for growth Temperature range for growt is Temperature range for growth is 15 to 40.degree. C. The optimum growth 15 to 40.degree. C. The optimum growth temperature ranges from 30 to temperature is 30.degree. C. 37.degree. C. The pH range for growth is 9.0 to The pH range for growth is 8.0 to 11.0. The optimum growth pH is 11.0. The optimum growth pH is similar to the above. pH 1O.0 to 11.0. Behavior to oxygen Aerophilic. Aerophilic. O-F test No growth is observed. No growth is observed. Assimilation of Assimilated are D-galactose, D-xylose, L-arabinose, lactose, glycerin, saccharides meribiose, libose, D-glucose, D-mannose, maltose, sucrose, **trehalose,** D-mannitol, starch, raffinose and D-fructose. Growth on a Grown at a salt concentration of 12%, but no growth at a salt salt-containing concentration of 15%. medium

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	472	sulfolobus or acidocaldarius	USPAT; US-PGPUB	2003/02/10 08:32
2	L2	4827	trehalose	USPAT; US-PGPUB	2003/02/10 08:33
3	L3	49	1 and 2	USPAT; US-PGPUB	2003/02/10 08:33
4	L4	128	non adj reducing adj saccharide$1	USPAT; US-PGPUB	2003/02/10 08:39
5	L5	21	1 and 4	USPAT; US-PGPUB	2003/02/10 08:40

PGPUB-DOCUMENT-NUMBER: 20020164723

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164723 A1

TITLE: Method of producing saccharide preparations

PUBLICATION-DATE: November 7, 2002

US-CL-CURRENT: 435/96

APPL-NO: 09/ 908395

DATE FILED: July 18, 2001

RELATED-US-APPL-DATA:

child 09908395 A1 20010718 parent continuation-of 09632392 20000804 US GRANTED parent-patent 6303346 US child 09632392 20000804 US parent continuation-of 09499531 20000210 US GRANTED parent-patent 6136571 US child 09499531 20000210 US parent continuation-of 09198672 19981123 US GRANTED parent-patent 6129788 US child 09198672 19981123 US parent continuation-in-part-of 09107657 19980630 US ABANDONED child 09107657 19980630 US parent continuation-in-part-of 08979673 19971126 US ABANDONED

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/632,392, filed on Aug. 4, 2000, now allowed, which is a continuation of U.S. patent application Ser. No. 09/499,531, filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. patent application Ser. No. 09/198,672, filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. patent application Ser. No. 09/107,657, filed on Jun. 30, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/979,673, filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference. [0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. [0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

PGPUB-DOCUMENT-NUMBER: 20020102696

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020102696 A1

TITLE: RECOMBINANT THERMOSTABLE ENZYME WHICH FORMS NON-REDUCING SACCHARIDE
FROM REDUCING AMYLACEOUS SACCHARIDE

PUBLICATION-DATE: August 1, 2002

US-CL-CURRENT: 435/200,435/183

APPL-NO: 09/ 419305

DATE FILED: October 15, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09419305 A1 19991015 parent continuation-of 08505448 19950721 US PATENTED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	190183/1994	1994JP-190183/1994	July 21, 1994
JP	189706/1995	1995JP-189706/1995	July 4, 1995